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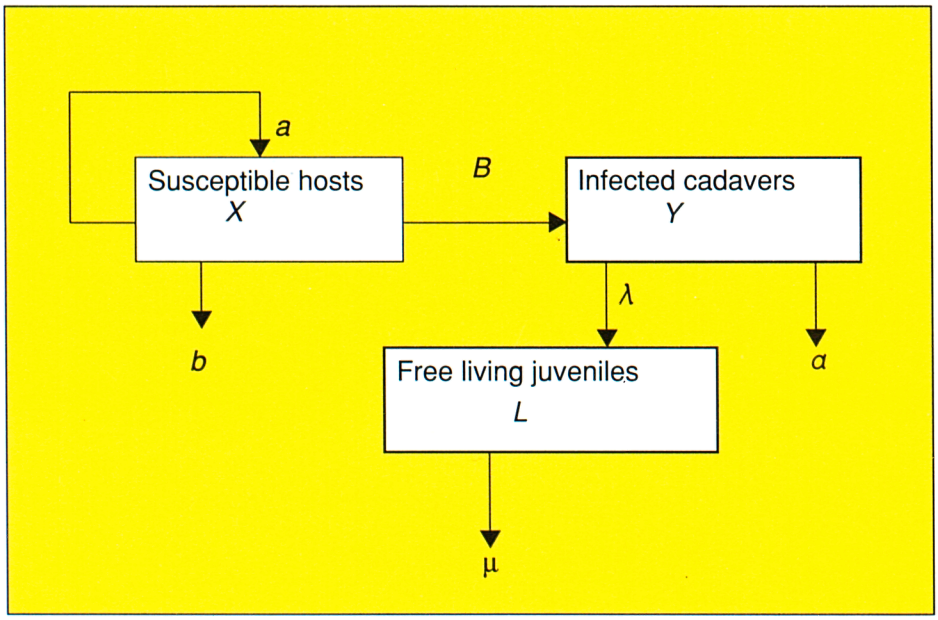
BIOTECHNOLOGY

Ecology and transmission strategies of entomopathogenic nematodes



Report

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Cover illustration

A schematic representation of the life cycle of entomopathogenic nematodes illustrating basic birth and death rates used in a generalized analytical model. (Courtesy of Peter Hudson and Rachel Norman.)

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Ecology and transmission strategies of entomopathogenic nematodes

Edited by
C.T. Griffin,
R.L. Gwynn,
J.P. Masson

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FOREWORD

The objective of COST Action 819 "Entomopathogenic Nematodes" is to enhance the use of these antagonists in biological control of soil-borne insect pests by coordination of European scientific expertise. Within the Action five working groups concentrate on isolation and identification, application and production, nematode biology and genetics, symbiotic bacteria and pathogenicity mechansims, and nematode ecology. This contribution is the results of a workshop held at the Kossuth L. University of Science in Debrecen, Hungary, from June 3 to 5, 1994, which was organised by the latter working group.

The use of entomopathogenic nematodes is still restricted to high value crops. Two ways can lead to a wider application in agriculture: The decrease of the production costs by means of modern biotechnology and the reduction of the applied nematode density. To obtain satisfactory control results about half a million nematodes are released per square meter, of which only very few reach the target insect. This raises the questions: What happens to the rest, and can the nematode application density be reduced if we were able to get them nearer to the target host or increase their persistence in the soil environment? Presently, we know very little about the ecological conditions influencing nematode population dynamics and about nematode behaviour in the soil environment. This first COST workshop on nematode ecology gained from expert contributions on soil ecology and nematode antagonists, on sampling methods and transmission strategies. It summarized field and laboratory data of COST participants on foraging behaviour, infection strategies and genetic diversity in entomopathogenic nematode populations. New questions were raised and innovative directions proposed for future orientation of research in the field of nematode ecology. This workshop was not aimed at directly answering the question how we can make maximum use of the nematodes' control potential and increase the field efficacy. Its main objective was to intensify discussion among COST participants and benefit from related scientific expertise in order to develop improved concepts on nematode ecology. We have made a good start and future COST workshops will step by step bring us nearer to the final goal of decreasing nematode application density.

This workshop was held in the pleasant and stimulating atmosphere of the Kossuth University. Thanks to the local organizers Attila Szentirmai and Andras Fodor for inviting us to Hungary and also to the working group co-ordinators Christine Griffin and Roma Gwynn who managed to bring together an overall excellent group of speakers, to whom we also owe our gratitude for their contributions. I also want to thank our sponsors who contributed to the success of the workshop: The Hungarian National Committee for Technical Development (OMFB), the European Union and special thanks to our COST secretary Jean-Pierre Masson, the International Organisation of Biological Control (IOBC) Working-Group "Insect Pathogens and Insect Parasitic Nematodes" and its convenor Peter Smits, Biosys (Palo Alto, USA), Ecogen Europe (Todi, Italy), Ecogen Germany (Raisdorf) and Koppert (Berkel en Rodenrijs, The Netherlands). With their financial support our sponsors have underlined their interest in our research activities and made possible the organization of this very fruitful workshop.

Raisdorf, January 12, 1995

Ralf-Udo Ehlers
(Chairman COST 819)

CONTENTS

POSTER ABSTRACTS

SURVIVAL OF ENTOMOPATHOGENIC NEMATODES IN SOIL

Frans A.A.M. de Leij

School of Biological Sciences, University of Surrey,
Guildford, Surrey, GU2 5XH, England.

INTRODUCTION

In this short review a conceptual model of the survival of entomopathogenic nematodes in the environment is put forward. Despite a considerable amount of research, generalisations about which factors determine survival and reproduction of entomopathogenic nematodes in natural soil lack consistency (Kaya, 1990; Richardson and Grewal, 1994). Furthermore, information about how these nematodes survive epizootically is almost completely absent. However, a good understanding of the ecology and behaviour of entomopathogenic nematodes in natural ecosystems is of prime importance if improvements in the field application of these nematodes are to be made (Gaugler, 1988).

Despite the lack of experimental data on the behaviour of natural populations of entomopathogenic nematodes in soil, some general ecological principles can be applied to the biological characteristics of these nematodes for the development of conceptual ecological models. Subsequently, such concepts can be used to improve the application of entomopathogenic nematodes in the field for the purpose of biological pest control.

IMPORTANCE OF SOIL CONDITIONS FOR NEMATODE SURVIVAL AND ACTIVITY

Disregarding soil conditions which are detrimental for nematodes, such as high levels of toxins, prolonged periods of drought or extended periods of anaerobic conditions, nematode activity is directly dependant on factors such as temperature (Kung *et al.*, 1990), soil moisture (Womersley, 1990) soil texture (Ames and Smart, 1989) and oxygen (Baxter and Blake, 1969). Waterlogging, dry conditions, low temperatures or lack of oxygen forces free living nematodes into a state of suspended activity. Only when conditions that are favourable for nematode activity are restored, can movement, feeding and reproduction be resumed. Although different nematode species might have different temperature optima, nematode activity is in general greatest in warm, moist, well aerated, coarse textured soils.

SURVIVAL OF ENTOMOPATHOGENIC NEMATODES IN SOIL

Like all nematodes that inhabit the soil, the dauer juveniles of entomopathogenic nematodes are dependant on favourable soil conditions for activity and infectivity. However, entomopathogenic nematodes are obligate parasites which are for their survival also dependant on their ability to locate and invade a susceptible insect host.

Therefore, the life strategy of the non-feeding dauer juveniles must be a compromise between the need to find a suitable host and long term survival in soil. Even under soil conditions which are optimal for nematode activity, the behaviour of these nematodes should optimise the cost of activity and the benefit of finding a suitable host. The most obvious costs of movement are the expenditure of the nematode's limited internal energy reserves and an increased chance of coming into contact with nematode antagonists (Timper *et al.*, 1991). To optimise their chance for survival and reproduction, dauer juveniles should combine minimal movement with maximal host encounter success. This is only possible if these nematodes employ a targeted host finding strategy (in contrast to random movement). There is some evidence that entomopathogenic nematodes orientate themselves in two ways towards a suitable host. In the first instance, species like *Steinernema carpocapsae* and *S. scapterisci* migrate to the soil surface (Campbell and Gaugler, 1993; Grewal *et al.*, this volume), while others migrate more deeply into soil and orientate themselves towards plant roots (Bird and Bird, 1986). Both the soil surface and the root environment are habitats that are richer in insects than the root free soil and the deeper soil layers. Subsequently, when a suitable host comes in close proximity to a dauer juvenile, the nematode will use chemical clues to locate its host (Akhurst, 1986; Grewal *et al.*, 1993). By employing a searching behaviour that is phased, nematodes can optimise their chance of encountering a suitable host, without unnecessary waste of internal resources.

REPRODUCTION STRATEGIES OF ENTOMOPATHOGENIC NEMATODES

The reproduction strategies employed by entomopathogenic nematodes can also give some vital clues about the behaviour of these nematodes in natural soil. Once a host is located and invaded the fecundity of each invading nematode is dependant on the number of invading individuals and the size of the host; a large host invaded by few nematodes will result in the highest fecundity. As a consequence, a population of related individuals that emerge from an insect cadaver should disperse to increase its chance of encountering a suitable host and to minimise the number of individuals that enter one host. Furthermore, dispersal increases the chance of matings between non-related individuals which is beneficial in terms of maintenance of genetic variability.

However, some *Steinernema* spp. seem to produce dauer juveniles that are either infective or non-infective, roughly in a ratio of three infective individuals to seven non-infective ones (Fan and Hominick, 1991; Bohan and Hominick, this volume). If it can be assumed that non-infective dauer juveniles will become infective over time, a population of entomopathogenic nematodes has an alternative mechanism, besides dispersal, at its disposal to avoid over-exploitation of available hosts. If the cost of dispersal is high, the solution of phased infectivity would be beneficial in terms of the reproduction success of a population of nematodes. Available evidence suggests that these nematodes have a patchy distribution in soil, either because they do not disperse from the cadaver (Spiridonov and Voronov, this volume), or because they migrate and concentrate in habitats that are favoured by suitable hosts.

The hypothesis that the distance that entomopathogenic nematodes migrate through soil is limited, implies that the soil environment as such is probably less important for the survival and reproduction of entomopathogenic nematodes than it is for non-

parasitic, free living nematode species. This hypothesis is to some extent supported for steinernematids by findings of Griffin *et al.* (1991), who found in a soil survey conducted in Ireland that steinernematids were recovered from all the soil types tested, and that there was no significant association between soil texture and frequency of recovery. Also, Mracek (1979) found in a soil survey in Czechoslovakia that as long as susceptible sawfly juveniles were present in forest soils, then neither the altitude of the forest nor the type of locality affected the distribution of *S. carpocapsae*. The last author has the opinion that availability of suitable hosts is the single most important factor that determines survival of these nematodes in soil.

SURVIVAL OF RELEASED ENTOMOPATHOGENIC NEMATODES

Regarding the apparent ability of entomopathogenic nematodes to survive in soil, the question remains why entomopathogenic nematodes decline more or less exponentially when applied to soil. To answer this question, a number of reasons are worth considering. Firstly, considerable inter- and intra-specific differences in tolerances of entomopathogenic nematodes have been demonstrated (Gwynn, 1993; Poinar, 1990). For example, Kung *et al.* (1990) showed that *S. carpocapsae* (the smallest *Steinernema* species) survived longer in sandy loam, which is fine textured, than *S. glaseri* (the largest *Steinernema* species). In sand, which is coarsely textured, the opposite was true. There are also large differences in temperature optima between different strains of entomopathogenic nematodes. Therefore, when entomopathogenic nematodes are applied to an environment different to that from which they were isolated, the chance of survival in this new habitat is likely to be reduced.

Another factor which might reduce nematode survival in soil, is the way these nematodes are normally applied to soil. When sprayed onto the soil surface, the nematodes will be exposed to UV radiation and rapid desiccation, both of which are detrimental (Gaugler and Boush, 1978). Survival of entomopathogenic nematodes could be improved by applying dauer juveniles to wet soil, immediately followed by irrigation which would help to wash the nematodes into the soil where they are less exposed to desiccation and UV radiation. Alternatively, nematodes can be injected into the soil which should give them some protection against desiccation and UV radiation.

A third factor which might determine nematode survival in soil is the way these nematodes are produced. The favoured way of producing them commercially is in liquid fermenters (Pace *et al.*, 1986). However, when fungal spores are produced in shake culture, they are larger and have thinner cell walls compared with those produced on solid media. Therefore, spores cultured in liquid culture are more vulnerable to adverse environmental conditions in the soil, than those produced on solid media. Although there is no evidence for this, it would not be surprising if nematodes produced in liquid culture might suffer the same problem; they might be more vulnerable to environmental extremes than nematodes that are naturally produced in soil.

Storage conditions before application might also be important. As is clear from the above, these nematodes are likely to survive in soil by being economical with their

energy reserves. If storage time and conditions allow the depletion of these reserves, their persistence will be negatively affected.

IMPROVING THE EFFICACY OF ENTOMOPATHOGENIC NEMATODES AS BIOLOGICAL CONTROL AGENTS

In order to improve the efficacy of entomopathogenic nematodes, it makes sense to try to match the application of these nematodes with their ecological adaptations as much as possible. Because of the previously mentioned genera, species and strain variability, there are possibilities for selecting strains that are optimally adapted to a certain environment with respect to temperature and soil type. This option is perhaps commercially not very attractive when carried out to an extreme; it means that a wide range of products have to be developed, each matched with a good promotion and advisory service.

Also the production of entomopathogenic nematodes might be improved. Part of the production process could be aimed at toughening the nematodes, so that they are able to resist adverse soil conditions, such as desiccation.

Thirdly, formulations might be improved. Instead of just spraying nematodes onto the soil surface, formulations that aim to increase nematode survival and attract insects might be highly effective. Seed coatings or small pellets containing nematodes as well as insect feeding stimuli and substances that would protect them from nematode feeding organisms are possibilities that could be worth considering.

And fourthly, applications of these nematodes can be improved. Applications that rely on the ability of entomopathogenic nematodes to disperse through soil may not be very effective. Injection of soil with nematodes might overcome some problems connected with UV radiation and rapid desiccation, but large volumes of soil will be free of nematodes if their capacity of dispersion is limited. Therefore, applications should not only be aimed at enhancing survival, but should also provide even distribution of the nematodes through soil. Alternatively, applications could be targeted to those places in the soil where insects are likely to cause damage, such as plant roots. Applications in the form of pellets or seed coatings, each containing 100-200 nematodes/pellet or seed, seem ideal.

CONCLUSIONS

Although more research is needed to evaluate how entomopathogenic nematodes survive in soil, there are strong indications that most species of entomopathogenic nematodes have adopted a sessile life strategy, aimed at conserving internal energy reserves. If this is the case, entomopathogenic nematodes should be less susceptible to adverse soil conditions than is so far thought. Their failure to survive in soil when applied to agricultural soils for the purpose of biological control of insect pests is therefore not so much governed by the soil environment, but more to a failure to select, produce, formulate and apply entomopathogenic nematodes in ways that take their natural ecological and biological adaptations into account.

REFERENCES

Akhurst, R.J. (1986). Controlling insects in soil with entomopathogenic nematodes. In: R.A. Samson, J.M. Vlak and D. Peters (Eds), *Fundamental and Applied Aspects of Invertebrate Pathology*, pp. 265-267. Proc. 4th Int. Colloq. Invertebr. Pathol., Wageningen, The Netherlands.

Ames, L.M. and Smart, G.C. Jr. (1989). Migration of an entomopathogenic nematode, *Steinernema* n. sp., through selected Florida soils. J. Nematol. **21**, 550.

Baxter, R.I. and Blake, C.D. (1969). Oxygen and the hatch of eggs and the migration of larvae of *Meloidogyne javanica*. Ann. appl. Biol. **63**, 191-203.

Bird, A.F. and Bird, J. (1986). Observations on the use of insect-parasitic nematodes as a means of biological control of root-knot nematodes. Int. J. Parasitol. **16**, 511-516.

Campbell, J.F. and Gaugler, R. (1993). Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (*Heterorhabditidae* and *Steinernematidae*). Behaviour **126**, 155-169.

Fan, X. and Hominick, W.M. (1991). Efficiency of the *Galleria* (wax moth) baiting technique for recovering infective stages of entomopathogenic rhabditids (*Steinernematidae* and *Heterorhabditidae*) from sand and soil. Revue Nématol. **14**, 381-387.

Gaugler, R. (1988). Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agriculture, Ecosystems and Environment **24**, 351-360.

Gaugler, R. and Boush, G.M. (1978). Effects of ultraviolet radiation and sunlight on the entomogenous nematode, *Neoaplectana carpocapsae*. J. Invertebr. Pathol. **32**, 291-296.

Grewal, P.S., Gaugler, R. and Selvan, S. (1993). Host recognition by entomopathogenic nematodes: behavioral response to contact with host faeces. J. Chem. Ecol. **19**, 1219-1231.

Griffin, C.T, Moore, J.F. and Downes, M.J. (1991). Occurrence of insect-parasitic nematodes (*Steinernematidae*, *Heterorhabditidae*) in the Republic of Ireland. Nematologica **37**, 92-100.

Gwynn, R.L. (1993). Development of cold active nematodes for insect pest control. PhD thesis, University of Reading.

Kaya, H.K. (1990). Soil ecology. In: Gaugler, R. and Kaya, H.K. (Eds.) *Entomopathogenic Nematodes in Biological Control*, pp. 93-115. CRC Press, Boca Raton.

Kung, P.A., Gaugler, R. and Kaya, H.K. (1990). Soil type and entomopathogenic nematode persistence. J. Invertebr. Pathol. **22**, 401-406.

Mracek, Z. (1979). The distribution of steinernematid nematodes in Czechoslovakia. Proc. Int. Colloq. Invertebr. Pathol. Prague, 145-146.

Pace, G.W., Grote, W., Pitt, D.E. and Pitt, J.M. (1986). Liquid culture of nematodes. International Patent No. WO/01074.

Poinar, J.O. Jr. (1990). Taxonomy and biology of *Steinernematidae* and *Heterorhabditidae*. In: Gaugler, R. and Kaya, H.K. (Eds), *Entomopathogenic Nematodes in Biological Control*, pp. 23-61. CRC Press, Boca Raton.

Richardson, P.N. and Grewal, P.S. (1994). Entomopathogenic nematodes and the soil environment. In: Bhandari, S.C. and Somani, L.L. (Eds), *Ecology and Biology of Soil Organisms*, pp. 106-130. Agrotech Publishing Academy, Udaipur.

Timper, P., Kaya, H.K. and Jaffee, B.A. (1991). Survival of entomopathogenic nematodes in soil infested with the nematode-parasitic fungus *Hirsutella rhossiliensis* (*Deuteromycotina: Hyphomycetes*). Biological Control **1**, 42-50.

Womersley, C.Z. (1990). Dehydration survival and Anhydrobic potential. In: Gaugler, R. and Kaya, H.K. (Eds), *Entomopathogenic Nematodes in Biological Control*, pp. 117-137. CRC Press, Boca Raton.

THE POTENTIAL IMPACT OF NATURAL ENEMIES ON THE SURVIVAL AND EFFICACY OF ENTOMOPATHOGENIC NEMATODES

Brian Kerry

Entomology and Nematology Department, Rothamsted Experimental Station, Harpenden, Herts.,AL5 2JQ, England.

SUMMARY

The natural enemies of nematodes in soil are described and their potential for affecting the efficacy of commercial applications of entomopathogenic nematodes assessed. Applications of entomopathogenic nematodes represent changes of <10% in total nematode densities in soil. Although some effects on the activities of natural enemies have been recorded after application, it remains to be demonstrated whether these influence the efficacy of entomopathogenic nematodes. Experience with plant parasitic nematodes indicates that natural enemies in soil may react too slowly to short-term changes in nematode abundance and would not prevent the few entomopathogenic nematodes required to kill insects reaching their target but that they may reduce their long-term survival.

INTRODUCTION

Most work on the importance of the natural enemies of nematodes has concerned those which attack plant pests or those which cause diseases in livestock. Although in recent years some quantitative data on the effects of microbial agents on the population dynamics of specific nematode pests have been published, research has been largely descriptive and empirical. Little work has been done on the natural enemies of entomopathogenic nematodes, in particular those most widely applied commercially (*Steinernema spp.* and *Heterorhabditis spp.*) and comments in this review draw heavily on experience from research in the biological control of plant parasitic nematodes.

Some nematophagous bacteria and fungi are known to provide effective control of specific nematode pests (Kerry, 1987). Such natural control, which results from increases in the density of the indigenous antagonistic soil microflora, may be very effective but it is difficult to manipulate and is slow to establish. The application of biological agents for the control of nematode pests has, so far, provided variable results (Kerry, 1990). Although many organisms feed on nematodes or affect their behaviour and survival in soil (Stirling, 1991), no organism has been identified which will provide adequate control in a range of circumstances. Hence, the successful utilisation of nematode antagonists in management strategies for plant parasitic nematodes is dependent on their integration with other control measures. The impact of natural enemies on the efficacy and survival of entomopathogenic nematodes applied to soil is discussed in the light of these comments.

TYPES OF NATURAL ENEMIES IN SOIL

The wide range of natural enemies that attack nematodes (Table 1) have been assessed for their potential impact on nematode populations in soil (Kerry, 1987; Stirling, 1991). In recent years, much research has concerned the fungal parasites of the sedentary females and eggs of cyst and root-knot nematodes because of their

TABLE 1. Natural enemies which may attack entomopathogenic nematodes in soil.

Predators	Parasites
Protozoa	Viruses
Tardigrades	Rickettsias
Turbellarians	Bacteria
Nematodes	Microsporidia (Poinar, 1988)
Enchytraeids	Trapping Fungi (Poinar and Jansson, 1986)
Mites (Epsky *et al.*, 1988)	Endoparasitic Fungi (Timper *et al.*, 1991)
Collembola	

Selected references have been cited which refer to work on natural enemies of entomopathogenic nematodes specifically.

potential as biological control agents for these important pests. However, such fungi will not infect active nematodes, including the infective juveniles of entomopathogenic nematodes. For these nematodes to be destroyed, the natural enemy must be able to pursue and eat them in soil or produce a trapping device or an adhesive spore from which the nematode may be colonised. As the infective dauer juveniles of *Steinernema spp.* and *Heterorhabditis spp.* do not feed in soil, those fungi (such as most *Harposporium spp.*) whose infective spores must be ingested, are unlikely to infect these nematodes. Although several microorganisms are known to produce nematicidal metabolites in *in vitro* tests, their significance in soil is unknown.

The natural enemies with potential to affect the survival of entomopathogenic nematodes in soil are the predators, nematode trapping fungi, and parasitic bacteria and fungi that produce adhesive spores. Although most predators are able to feed on a wide range of soil nematodes, many do have host preferences and they differ in their dependence on nematodes as a food source. The ability to pursue prey in the pore spaces frequented by nematodes may limit the efficacy of predation. Nematodes do not create their own living space and are confined to films of moisture on soil particles in pores with apertures greater than their body width (about 25-50 µm for entomopathogenic nematodes). Hence, nematodes may escape if predators are too large to enter such pores; voracious feeders in simple laboratory tests have proved much less effective in soil. However, in potting composts or artificial soils which are less compacted than field soils, and in the clefts which frequently occur between potting media and containers, predators may be very active and have significant effects on nematode populations. Collembola and mites are the most numerous predatory arthropods in soils and their estimated numbers and predatory abilities suggest they

must have considerable influence on nematode densities, but this remains to be demonstrated for any nematode species in soil. Similarly, there is little information on the effects of other predators such as nematodes, protozoa and tardigrades on nematode abundance in soil.

Nematophagous fungi (Barron, 1977) continue to command most research interest amongst those working on nematode antagonists. Nematode trapping fungi are facultative parasites that develop saprophytically in soil and establish a mycelium on which a variety of trap structures are produced to ensnare nematode prey. These fungi form a very diverse group and differ markedly in their nutritional dependence on nematodes and in their trapping efficiency; some nematodes are much more easily captured than others and there is some evidence of host specificity amongst these fungi. Trapping activity is thought to be dependent on the organic matter status in soil rather than on the density of nematodes but there has been little work done on the ecology of these organisms in soil.

Fungi such as *Hirsutella rhossiliensis* and *Drechmeria coniospora* produce much smaller spores than the trapping fungi and these spores remain dormant in soil until they adhere to a nematode host. As *D. coniospora* failed to parasitise infective juveniles of *Steinernema spp.* or *Heterohabditis spp.* (Poinar and Jansson, 1986), *H. rhossiliensis* is the only nematophagous fungus on which some data have been collected on its effect on the survival of entomopathogenic nematodes (Timper *et al.*, 1991). Few spores attached to the cuticle of dauer juveniles unless the outer retained cuticle was removed. Hence, the second stage juvenile cuticle retained by the dauer stage may provide some protection from antagonists as well as reducing water loss in dry conditions. Spores attached to nematodes germinate and produce a penetration peg which breaches the cuticle and forms a post infectional bulb from which a mycelium develops throughout the nematode, which is normally killed within 24 hrs.

Pasteuria spp. are obligate parasites of a wide range of nematodes but individual species and populations of the bacteria have very restricted host ranges and, so far, an isolate that parasitises entomopathogenic nematodes has not been recorded. *Pasteuria penetrans* infects root-knot nematodes and has been studied more closely than the two other *Pasteuria* species that have been described. Highly resistant infective spores remain dormant in soil until they adhere to a passing second stage juvenile. The spores germinate after the nematode has entered a host root but before it has moulted to the next stage. The bacterium does not prevent the development of the nematode to adulthood but infected females fail to produce eggs and are eventually filled with a mass of spores which are released into the soil when the cadaver breaks down. There is no evidence of the involvement of toxins in the relationship and nematodes may continue to grow for 20 days after their infection, by which time they have become adult. In pot tests and in microplots, *P. penetrans* has proved the most effective microbial agent for the control of root-knot nematodes but failure to establish *in vitro* culture methods and its specificity have prevented commercial development. Again, the retained cuticle of the dauer juvenile of entomopathogenic nematodes may offer protection from this parasite.

NATURAL ENEMIES AND THE REGULATION OF NEMATODE POPULATIONS

The role of natural enemies in the regulation of nematode abundance remains unclear and such information as there is has largely resulted from a few studies on the influence of nematophagous bacteria and fungi on specific pests. Nematode antagonists appear slow to increase in soil to densities that are capable of regulating the populations of plant parasitic nematodes. Soils that suppress the multiplication of some nematode pests have only developed under perennial crops or crops grown in monocultures, when they may take at least four cropping cycles to become established. Long-term studies on the population dynamics of the cereal cyst nematode (Gair *et al.,* 1969) demonstrate that in suppressive soils the nematode declines to an equilibrium density of about 2-5 eggs/g soil (equivalent to 4-10 x 10^5 /m^2 to plough depth). Hence, the nematode is still numerous in soil even when below the damage threshold for cereal crops. For less tolerant crops or those susceptible to nematode-transmitted viruses where damage can be caused by very few nematodes, biological control agents are unlikely to be effective unless they are applied in very large numbers.

Some natural enemies that attack the juvenile stages of plant parasitic nematodes have reduced yield losses to crops but none has prevented nematode populations increasing. Only those parasites that kill the reproducing adult females have been able to control multiplication. In the case of the cereal cyst nematode such control occurred whilst the females remained aggregated on the root surface (Kerry *et al.,* 1982). Factors which influence the mobility of nematodes in soil greatly affect the acquisition of the non-motile spores of obligate nematophagous bacteria and fungi. Some of these relationships have been quantified and nematode spore burdens related to the density of spores in soil and the duration of the nematode's migration. Nematodes are more likely to encounter spores of *P. penetrans* (Stirling, 1984) or *H. rhossiliensis* (Jaffee *et al.,* 1990) when spore densities are large and temperature and moisture conditions promote movement of the nematodes in soil.

POTENTIAL EFFECTS ON ENTOMOPATHOGENIC NEMATODES

Total nematode populations in soil may reach densities of 30 x 10^6/m^2 and even under grass in the Arctic tundra there may be 7.5 x 10^6/m^2 (Yeates, 1981). Hence, normal applications (1 x 10^6/m^2) of entomopathogenic nematodes represent <10% increases in the densities of nematodes and the response of non specific antagonists, such as certain predators, in soil may be small. However, if these nematodes remain aggregated after application they may affect the activity of natural enemies including predators. Changes in the community structure of soil nematodes including an increase in predatory species following application of entomopathogenic nematodes have been recorded (Ishibashi and Kondo, 1986). Survival of entomopathogenic nematodes added to autoclaved soil was significantly greater than in non sterilised soil (Ishibashi and Kondo, 1986) and several natural enemies known to feed on these nematodes (Kaya, 1990) may affect their longevity. Although there is little doubt that natural enemies may reduce the survival of entomopathogenic nematodes, there is a need to demonstrate whether numbers are reduced sufficiently to influence the rates of infection of insects in soil.

Infective juveniles of *S. glaseri* are more active than the less migratory *S. carpocapsae* and encountered more spores of *H. rhossiliensis*, which may have caused the poorer survival of this species in non sterilised soil (Timper *et al.*, 1991). Also, differences in the adhesion of spores to nematode cuticle and in their germination were observed on different nematode species, and the protective value of the retained second stage juvenile cuticle in the dauer stage, which prevented attachment of fungal spores, was demonstrated. Active, infective juveniles in soil may increase the chances of locating an insect host but will also increase the probability of encountering non motile spores.

METHODS FOR MEASURING PARASITISM AND PREDATION BY NATURAL ENEMIES

Several direct and indirect methods have been used to assess the effects of antagonists on plant parasitic nematode populations and these could be used in similar studies on entomopathogenic nematodes (Table 2). Infected nematodes should be extracted from soil using the sugar flotation method (Jenkins, 1964) and not methods that rely on the nematodes being active, such as the Baermannn funnel, which are very inefficient. Soil sprinkle plates (Barron, 1977) have been widely used to assess the

TABLE 2. Methods for the estimation of the effects of nematode antagonists in soil.

Direct:		
	(a)	Extraction of diseased nematodes from soil
	(b)	Observations on soil-sprinkle plates
	(c)	Addition of selected natural enemies to soils where they are absent
Indirect		(methods to increase/decrease activity of natural enemies in soil and the subsequent changes in nematode populations measured):
	(a)	"Selective" chemical treatments
	(b)	Organic soil amendments

presence of nematode trapping fungi but they do not necessarily indicate the activity of these organisms in soil. The most useful method for assessing the role of individual natural enemies is to add the organism where it is absent and measure the survival of the nematode population in treated and untreated soil. Such methods depend on being able to obtain large numbers of each organism and to establish them in soil. Indirect methods may be easier to perform but they frequently provide data that are difficult to interpret because it is impossible to apply a treatment to soil and affect only one group of organisms. Hence, applying sufficient organic matter to soil to increase the activity of nematode trapping fungi also results in the release of breakdown

products, especially fatty acids, which are directly nematicidal, so reductions in nematode populations are not solely due to the activity of the fungi. Fungicides have been added to soil to reduce the densities of nematophagous fungi in soil and the subsequent increase in nematode densities used as an indicator of the importance of these antagonists in the regulation of nematode populations (Crump and Kerry, 1987). However, the use of selective pesticides is unlikely to affect only the target organisms. Convincing data on the influence of natural enemies on the numbers of entomopathogenic fungi can only be provided by a combination of these methods.

CONCLUDING COMMENTS

In the same way that natural enemies may reduce but not prevent the invasion of plant roots by parasitic nematodes it seems unlikely that they will prevent the few nematodes required to kill an insect reaching their target, especially as inundative treatments are used to obtain quick kills of insect pests. Most commercially produced nematodes fail to survive longer than 4 weeks after application to soil (Georgis, 1992) and epizootics are rarely established. However, some recycling of nematodes released from cadavers may occur. Natural enemies tend to respond slowly to changes in nematode densities and so are more likely to reduce the longterm survival of nematodes in soil than affect the efficacy of inundative treatments. However, quantitative data on the influence of natural enemies on the abundance of entomopathogenic nematodes should be obtained if application rates are to be reduced without loss of efficacy.

REFERENCES

Barron, G.L. (1977). *The Nematode Destroying Fungi.* Canadian Biological Publications, Guelph.

Crump, D.H. and Kerry, B.R. (1987). Studies on the population dynamics and fungal parasitism of *Heterodera schachtii* in soil from a sugar-beet monoculture. Crop. Prot. **6**, 49-55.

Epsky, N.D., Walter, D.E., and Capinera, J.L. (1988). Potential role of nematophagous microarthropods as biotic mortality factors of entomogenous nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). J. Econ. Entomol. **81**, 821-825.

Gair, R., Mathias, P.L., and Harvey, P.N. (1969). Studies of cereal cyst nematode populations and cereal yields under continuous or intensive culture. Ann. appl. Biol. **63**, 503-512.

Georgis, R. (1992). Present and future prospects for entomopathogenic nematode products. Biocon. Sci. Tech. **2**, 83-99.

Ishibashi, N., and Kondo, E. (1986). *Steinernema feltiae* (DD-136) and *S. glaseri*: Resistance in soil and bark compost and their influence on native nematodes. J. Nematol. **18**, 310-316.

Jaffee, B.A., Muldoon, A.E., Philips, R., and Mangel, M. (1990). Rates of spore transmission, mortality and production for the nematophagous fungus *Hirsutella rhossiliensis*. Phytopathology **80**, 1038-1088.

Jenkins, W.R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. **48**, 692.

Kaya, H.K. (1990). Soil Ecology. In: Gaugler, R. and Kaya, H.K. (Eds.) *Entomopathogenic Nematodes in Biological Control,* pp. 93-116. CRC Press, Boca Raton.

Kerry, B.R. (1987). Biological Control. In: Brown, R.H. and Kerry, B.R. (Eds.) *Principles and Practice of Nematode Control in Crops,* pp. 233-263. Academic Press, New York.

Kerry, B.R. (1990). An assessment of progress toward microbial control of plant parasitic nematodes. J. Nematol. **22**, 612-631.

Kerry, B.R., Crump, D.H. and Mullen, L.A. (1982). Studies of the cereal cyst nematode, *Heterodera avenae* under continuous cereals, 1975-1978. II. Fungal parasitism of nematode eggs and females. Ann. appl. Biol. **100**, 489-499.

Poinar, G.O. (1988). A microsporidian parasite of *Neoaplectana glaseri* (Steinernematidae: Rhabditida). Rev. Nematol. **11**, 359-361.

Poinar, G.O. and Jansson, H.B. (1986). Infection of *Neoaplectana* and *Heterorhabditis* (Rhabditida: Nematoda) with the predatory fungi, *Monacrosporium ellipsosporum* and *Arthrobotrys oligospora* (Moniliates: Deuteromycetes). Rev. Nematol. **9**, 241-244.

Stirling, G.R. (1984). Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. Phytopathology **74**, 55-60.

Stirling, G.R. (1991). *Biological Control of Plant Parasitic Nematodes.* CABI, Wallingford.

Timper, P., Kaya, H.K. and Jaffee, B.A. (1991). Survival of entomogenous nematodes in soil infested with the nematode-parasitic fungus *Hirsutella rhossiliensis* (Deuteromycotina: Hyphomycetes). Biol. Con. **1**, 42-50.

Yeates, G.W. (1981). Nematode populations in relation to soil environmental factors: a review. Pedobiologia **22**, 312-338.

ANALYSIS OF SPATIAL VARIABILITY IN PEST MANAGEMENT

Wopke van der Werf[1], Tom Been[2] & Corné G. Kocks[3]

[1]Wageningen Agricultural University, Department of Theoretical Production Ecology, P.O. Box 430, 6700 AK Wageningen, The Netherlands; [2]Research Institute of Plant Protection, IPO-DLO, P.O. Box 9060, 6700 GW Wageningen, The Netherlands; [3]Wageningen Agricultural University, Department of Phytopathology, P.O. Box 8025, 6700 EE Wageningen, The Netherlands

SUMMARY

Spatially implicit and explicit techniques are used for describing aspects of spatial variability of pests, natural enemies, diseases, plants (weeds) and other biotic and abiotic factors in agricultural fields and natural terrain. The choice of technique depends upon the purpose of the study and the possibility to gather the required data. We present basic concepts underlying geostatistical analysis of disease patterns and the description of sampling and monitoring processes with probability distributions and other models. The mathematical methods are used for designing efficient pest sampling protocols. This is illustrated with case studies on the spatial pattern of a bacterial disease in cabbages, the design of monitoring systems for pest mites in apple and the detection of cyst nematode patches in potato.

INTRODUCTION

Densities of pests and their natural enemies (e.g. entomopathogenic nematodes) vary over space. Insight in the spatial dimension of pest attack is required when developing reliable and efficient methods for detecting pest presence and determining the average density. Spatial patterns may suggest the mechanisms underlying the introduction and spread of disease or pest in a field. Control measures may be targeted to hot spots, where density is highest.

Observations on the density (or some other expression of 'presence') of a pest or disease can be collected in two fundamentally different ways; *with* or *without* the spatial coordinates of the observation. In the first case, a map can be drawn of pest density in space and techniques may be used to analyse and describe the spatial pattern. In the second case, the result of the observations is a collection of numbers. Analysis and description then focus on the frequency distribution of these numbers, disregarding the spatial coordinates. When spatial coordinates are not recorded, the data become spatially implicit, i.e. spatial relationships cannot be retrieved from the

data set, although they still underly its statistical attributes. When spatial coordinates are retained, the resulting spatial analysis and relationships are *explicit.* Spatially implicit and explicit techniques are both used in research on pest ecology, but for different purposes. Spatially explicit techniques are primarily used for describing and mapping disease patterns and studying mechanisms underlying the initiation and spread of disease in field crops. A practical application of such studies is the derivation of optimal sampling distances and patterns. Spatially implicit techniques are used for describing, analysing and predicting the statistical properties of sampling methods and for developing sampling methods that strike an optimal balance between sampling effort and sampling accuracy.

The next section of this paper is methodological. We present here some important concepts in the analysis of spatial variability in pest management. The application of these concepts is illustrated in a section with three case-studies. The overall aim of this presentation is to highlight approaches that are potentially useful and can be easily adapted for the study of entomopathogenic nematodes.

METHODOLOGY

Principles of geostatistical analysis

Two approaches to *explicit* spatial analysis of pest and disease patterns dominate the phytopathological literature. One is based on geostatistics (Burrough, 1987) while the other is based on auto-regressive integrated moving average (ARIMA) models (Hudelson *et al.,* 1989). Geostatistics was originally developed for spatial interpolation and mapping in geology and mining. It is now widely used in soil science. Spatial autocorrelation analysis evolved from time series analysis. Despite their different origin, terminology and calculation methods, the two approaches have several conceptual similarities. Geostatistics is becoming an accepted technique for mapping disease patterns (Chellemi *et al.,* 1988; Lannou & Savary, 1991; Munkvold *et al.,* 1993; Nelson et al., 1994; Stein *et al.,* 1994).

The purpose of geostatistics is to create a (contour)map of the spatial pattern of a spatially varying characteristic, using interpolation between observed data points. The interpolation is done in such a way that the obtained estimates are unbiased and have minimum variance. The first step in a geostatistical analysis is the description of the statistical relationship between data points as a function of their distance. (As a rule, the correlation diminishes with distance.) The statistical descriptor of (un)relatedness, used in geostatistics, is the semi-variance:

$$\gamma(h) = \frac{1}{2} E[Z(x+h)-Z(x)]^2$$

where

$\gamma(h)$	is the semi-variance for a spatial distance h
$Z(x)$	is the value for a characteristic (say pest density) at a location x
E	denotes the statistical expectation

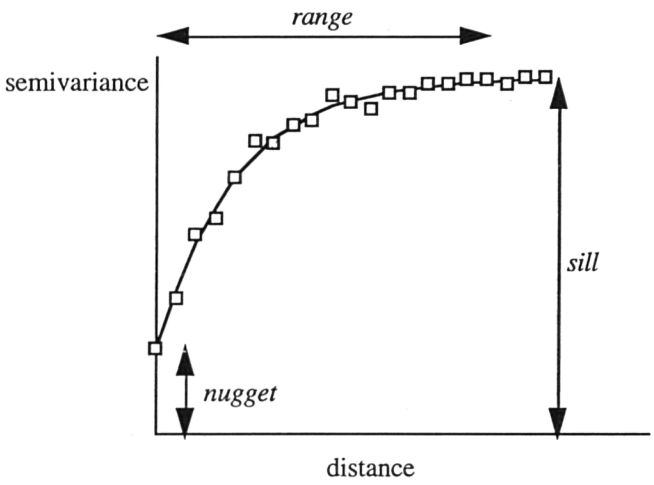

Fig. 1 Semivariogram. Horizontal axis denotes spatial distance, vertical axis the semi-variance, which is a measure of the average squared difference between observations made at a given distance. Points are calculated from a spatially indexed data set. The drawn line is a non-linear regression equation.

In a data-set of observations, collected at N different sites in a field, there are theoretically $N(N-1)/2$ estimates of the semi-variance. The data are grouped in distance classes and the semi-variance for a distance class is plotted against the distance (Fig. 1). The resulting figure is usually a curve that starts at a non-zero value for distance 0 (the *nugget*) and increases in a nonlinear way to a maximum value (the *sill*). The *range* is a measure for the distance over which the semi-variance is (substantially) smaller than the sill. A smooth curve is drawn through the data points, using nonlinear regression with an appropriate function (Fig. 1). A variety of functions describing semivariograms is used. One of those is the negative exponential:

$$\gamma(h) = nugget + (sill - nugget)(1 - \exp(-h/range))$$

When there is no spatial interdependence, the semivariogram becomes a horizontal line. This is called the *pure nugget effect*.

There are some requirements when calculating semivariograms. First, there should be at least 50 points per distance class. Second, not all the $N(N-1)/2$ data pairs may be used for the calculation of the semi-variance because the largest distance appearing in the semi-variogram should not exceed approximately half of the length of

the field. Otherwise only extreme parts of the field would be involved in the calculation, so that the result can not be regarded as representative for the whole. When constructing semivariograms, it is assumed that the semi-variance is a function of distance only, and that the variance is constant over space. Violations of these assumptions can be solved by a.o. transformation of data, using moving averages, or representing large scale variation in the underlying mean by fitting a spatial response surface (Burrough, 1987).

Spatial interpolation between observation points is done with a technique called *kriging* after one of its developers, the South African mining engineer D.G. Krige. Kriging estimates are a linear sum of weighted observations within a certain neighbourhood:

$$\hat{Z}(x_0) = \sum_i w_i Z(x_i)$$

where $\hat{Z}(x_0)$ is the interpolated function value at location x_0 and w_i is the weight of the i th measurement $Z(x_i)$ The weights depend on the semivariogram. They can be positive or negative and their sum is 1. They are determined such that the kriging estimate of $\hat{Z}(x_0)$ is unbiased and has minimum variance. The actual accuracy of $\hat{Z}(x_0)$ depends on the shape of the semivariogram, and on the density and spatial pattern of the observations.

With use of kriging, it is possible to predict values at unvisited locations. This prediction is based on neighbouring observations and the configuration of these observations. Kriging finally yields a (contour)map of estimated values and their associated prediction errors. More detailed information is given by Journel & Huijbregts (1978).

Principles of sampling and monitoring

Interest in pest sampling was spawned by the concept of Integrated Pest Management, which emerged in the Western world in the 1960s as a reaction to the alarming side-effects of chemical pest control during the 1950s. It was felt that Integrated Pest Management should be primarily based on cultural and biological controls while chemical control should only be used as an 'emergency break' in those cases in which these natural controls failed. The concept of the economic damage threshold was coined to establish whether pest density was more damaging than the cost of treatment (Stern *et al.*, 1959). Methods were required to determine efficiently whether a pest population density was above or below this threshold. This decision problem requires a sampling methodology that results in a *classification* of density (Binns, 1994). A classification procedure may be designed such that the probability of a misclassification for densities deviating a specified amount from the threshold does not exceed a tolerance value (Binns, 1994; see below). Pests and diseases that pose a risk during a whole growing season may require repeated sampling through time.

Efficient monitoring can be achieved by linking classification procedures in time (Nyrop *et al.*, 1994; *cf.* second case study).

A special case of classification is *detection.* For instance, it may be asked whether a nematode species occurs in a piece of land or not. For a detection procedure, it is important to state explicitly the probability of a classification as nematode-free, when in fact the species is present (*cf.* third case study). This probability of misclassification decreases with nematode density. It depends also on the spatial aggregation of the nematodes and the sampling pattern. *Estimation* of density is often the appropriate objective in research (Wilson *et al.*, 1989), e.g. when describing the occurrence and dynamics of an organism in time and space. The *precision* of an estimate can be expressed as a standard error or as a variation coefficient. Density estimates (of sufficient accuracy) are inputs for *maps* showing spatial trends and patterns.

Table 1 purposes of sampling

technique	question/purpose
estimation	research on population dynamics or spatial pattern
detection	production of certified nematode-free seed potatoes
classification	intervene or not at a specific stage of pest phenology or crop growth
monitoring	intervene or resample later during a whole cropping season
mapping	visualizing spatial trends, patterns and relationships in pest density

One of the theoretical cornerstones of practical sampling programs in pest management is the description of the frequency distributions of observed pest and disease densities by means of statistical relationships (e.g. Taylors Power Law) and probability models (e.g. the negative binomial distribution). These mathematical tools are derived from an analysis of observations, in which the spatial dimension is neglected. This neglect is warranted when the interest of a grower or scout is in the overall density and its effect on crop productivity and quality and not in the spatial pattern. Some of the most important mathematical tools are presented. The use of these tools is illustrated in a case study on the development of a monitoring plan, based on sequential classification sampling plans.

Basic tools for describing sampling distributions

The spatial pattern, the sample size and the spatial distribution of samples affect the frequency distribution of sample counts (sampling distribution). If the location of each damaging organism were independent of that of the other, the spatial distribution would be random. For any size of sample and spatial arrangement of samples, the resulting frequency distribution can then be described by the Poisson probability

distribution, which is characterized by a single parameter, the average density (μ).

$$P_x = e^{-\mu} \frac{\mu^x}{x!}$$

For the Poisson distribution, the variance of density is equal to the average density.

$$\sigma^2 = \mu$$

Subsequent probabilities of the Poisson distribution are calculated by

$$\begin{cases} P_0 = e^{-\mu} \\ P_{x+1} = \dfrac{\mu}{x+1} P_x \end{cases}$$

As a rule, however, pests and diseases deposit their offspring close to themselves, resulting in patchy distributions. Such spatial patterns result in frequency distributions with longer tails than the Poisson distribution. The negative binomial distribution is widely used (though not the only usable function) for describing these long-tailed frequency distributions. The negative binomial distribution is defined by:

$$P_x = \left(\frac{k}{k+\mu}\right)^k \binom{k+x-1}{x} \left(\frac{\mu}{k+\mu}\right)^x$$

The parameter *k* is called the dispersion parameter. The variance of the distribution and the length of the tail *decrease* with *k* (for given μ). For large *k*, the negative binomial distribution is similar to the Poisson distribution. For the negative binomial distribution, the variance of density is greater than the mean:

$$\sigma^2 = \mu\left(1 + \frac{\mu}{k}\right)$$

Probabilities of the negative binomial distribution are calculated with

$$\begin{cases} P_0 = \left(\dfrac{k}{k+\mu}\right)^k \\ P_{x+1} = \dfrac{k+x}{k+\mu} \dfrac{\mu}{x+1} P_x \end{cases}$$

The negative binomial distribution fits many observed frequency distributions because it has a quite flexible shape, ranging from the often bell-shaped low variance Poisson distribution (k -> ∞) to the monotonously decreasing high variance geometric distribution ($k = 1$) and beyond ($k < 1$).

In observed data sets, the parameter k usually has some relationship to the mean. This relationship can often be described with Taylors Power Law, which draws a linear relation between log(variance) and log(mean). For instance, for red mites on apple leaves, Nyrop & Binns (1991) used the relationship

$$\log\left(\hat{\sigma^2}\right) = \log(4.27) + 1.37 \log(\mu) \quad \text{or} \quad \hat{\sigma^2} = 4.27\,\mu^{1.37}$$

Using the relationship $k = \dfrac{\mu^2}{\sigma^2 - \mu}$

k can be estimated from the mean of the distribution.

Based upon a fitted probability distribution, the probability of the zero class can be used to estimate the relationship between the average density and the proportion of occupied sample units. This relationship can also be fitted with an empirical relationship

$$\ln\left(-\ln\left(1-p_T\right)\right) = a + b\ \ln(\mu)$$

Here p is the proportion of sample units with more than T specimens of the damaging organism, μ is average density, and a and b are regression parameters.

Sequential classification sampling

For the question whether a pest density or incidence is below or above a threshold, Walds Sequential Probability Ratio Test (SPRT) provides an optimal decision procedure. Instant recipes (and spreadsheets that do the calculations) are available to construct an SPRT-based sampling procedure for a range of sampling distributions, including the Poisson, negative binomial, binomial and normal distribution (Fowler & Lynch, 1987). For the negative binomial distribution, the following information is required and sufficient to construct an SPRT.

1. Information defining sampling performance
   The probability α of erroneously deciding that the average density is above the threshold T, when the actual density is in reality μ_0, which is smaller than T.
   The probability β of erroneously deciding that the average density is below the threshold T, when the actual density is in reality μ_1, which is greater than T.
2. Information defining the sampling distribution
   The dispersion parameter k

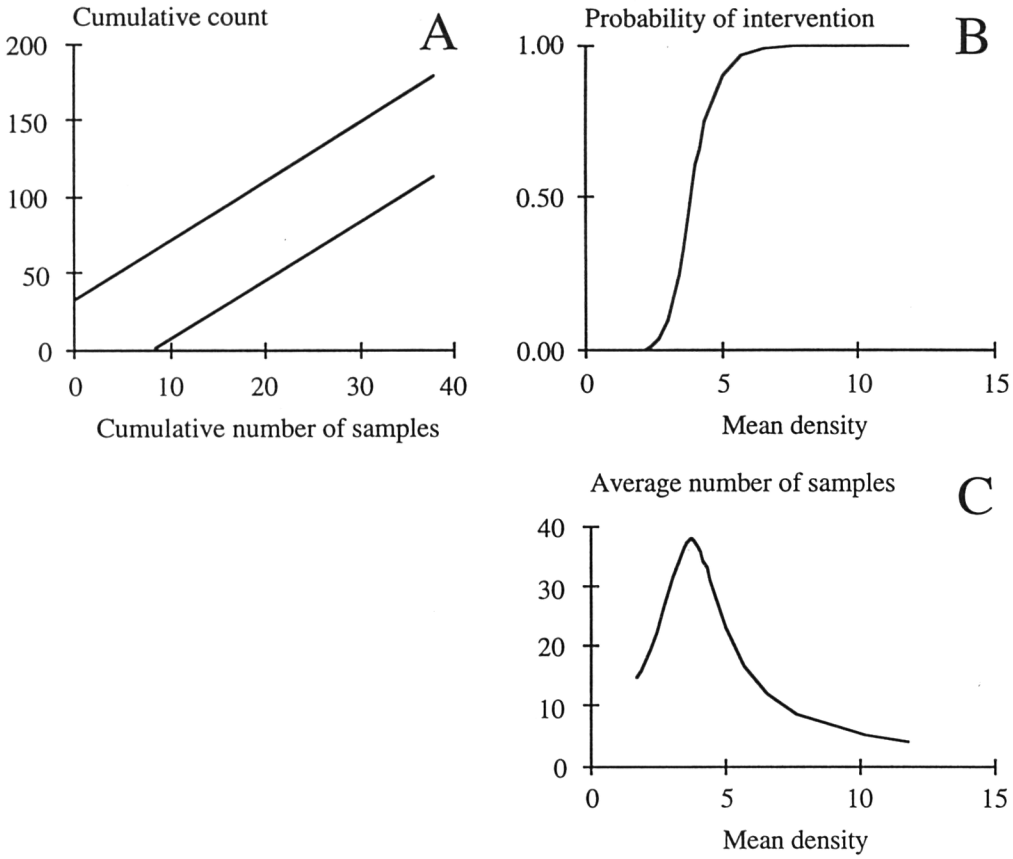

Fig. 2 (A) Stoplines of a sequential sampling plan, based on Walds Sequential Probability Ratio test; (B) Probability of intervention, as a function of the mean density; (C) Average number of samples required to make a decision, as a function of the mean density. This specific example was generated using a negative binomial distribution with $k = 0.6$; $\mu_0 = 3.0$; $\mu_1 = 5.0$; $\alpha = \beta = 0.1$.

The sequential plan is executed by inspecting sample units one by one and plotting the cumulative count against the cumulative number of sample units in Fig. 2A. When one of the two stoplines is crossed, sampling is terminated. When the higher stopline is crossed, the decision is to intervene. When the lower stopline is crossed, the decision is not to intervene.

The performance of a sequential sampling plan is judged by the average number of samples and the probability of intervention, which are both functions of the true mean density. The probability of intervention (Fig. 2B) is an increasing function of density, with the 50% point close to the threshold *T*. The average number of samples (Fig. 2C) is a maximum function. The highest number of samples is required (on average) when

the actual density is close to the threshold, because the probability of remaining between the stoplines is then greatest. The expected number of samples decreases as true density is further removed from the threshold. The two probability statements that define the SPRT, mark the position of two points on the probability of intervention-function of Fig. 2B: namely the points (μ_0, α) and $(\mu_1, 1-\beta)$. As a rule, μ_0 and μ_1 are chosen such that the threshold T is the average of them, while the error rates α and β are equal.

Steps in developing a monitoring protocol

When a pest must be monitored during an extended period of time, a procedure is required that ensures timely intervention when required, but that at the same time limits the frequency of observations as much as warranted. As an example of the practical application of probability and dynamical models, a guideline for developing a monitoring protocol is given. Similar guidelines may be developed for other sampling techniques, as classification, detection or estimation.

Step 1 Collect a data set defining the range of possible spatial distributions (irregularity in space, patchiness, variability), population dynamics (outbreaks, steady state, biological control) and the effect of the pest on growth and yield. This is the basic data set.

Step 2 Describe the sampling process, population dynamics and pest damage with mathematical models. These are basic models that serve as tools in the construction and evaluation of the monitoring protocol.

Step 3 Devise a monitoring protocol, using the basic models to take account of the spatial distribution, population dynamics and growth reducing effect of the pest or disease. The monitoring protocol provides decision support on when to make observations and whether or not to intervene.

Step 4 Simulate usage of the monitoring protocol. Calculate performance characteristics taking account of uncertainties in the outcome of sampling and in the dynamics of the pest by using stochastic parameters in the basic models.

Performance characteristics of a monitoring protocol are:
- total number of sampling occasions ± SD
- total number of samples ± SD
- overall probability of intervention
- cumulative pest density over time ± SD
- pest density at intervention ± SD

The first two variables are indices of effort while the others quantify the quality of control. Performance characteristics depend on spatial variability and pest dynamics

Step 5 Re-iterate steps 3 and 4, until an acceptable performance is attained. If no acceptable performance can be attained, consider developing alternative protocols for specific situations, for instance with and without natural enemies and for cold and warm weather.

Step 6 Test an acceptable monitoring protocol under field conditions.

Step 7 Apply the well-tested monitoring protocol in practice. Obtain feedback from users and continuously improve the protocol.

Variance components

A technique that is sometimes useful in defining sampling programs is analysis of variance and estimation of variance components. For instance, Nyrop & Binns (1991) discuss the contribution of between-tree-variation and within-tree-variation to total sampling uncertainty for a leaf miner species in apples. Based upon this analysis, it was concluded that it was more cost effective to inspect many trees and only few branches per tree than to sample many branches on a few trees. In the later case, the comparatively large variance component between-trees was not compensated for by adequate repetition. Based on an estimation of variance components and quantification of the costs of sampling trees and branches within trees, the most accurate sampling scheme for given cost could be defined. Alternatively, the cheapest scheme providing a minimum precision could be identified.

CASE STUDIES

Case: geostatistical analysis of black rot patterns in cabbage

The use of geostatistics is illustrated with data from black rot in cabbage. Black rot is caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939. The bacterium is seed-borne, and infested seed is an important source of inoculum. Cruciferous weeds, plant residue and cabbage volunteers are other inoculum sources. The pathogen can spread rapidly with wind and rain storms. The main objective of the study was to determine how far sampling intensity and observation time could be reduced, without obtaining a 'blurred' image of the spatial disease pattern.

A natural black rot epidemic was studied in a 20 by 20 m red cabbage field near Wageningen. The field had been planted with 1600 red cabbage plants in a 50 x 50 cm square arrangement on 15 May 1990. Rows ran northeast. Black rot incidence was scored visually on all 1600 plants on 24 August. This complete inventory is referred to as sampling plan I. To determine sampling with reduced intensity, geostatistical

mapping of the disease pattern in the plot was performed on the basis of reduced data sets. One set (referred to as sampling plan II) uses the presence/absence data on every third plant (yielding 533 data points). The third plan uses data on one in five plants (320 observations).

Semivariograms were calculated and fitted for each sampling intensity (Fig. 3). Exponential models gave the best fit. The calculated range for the three sampling intensities differed only slightly, varying from 2.7 with plan I to 2.2 m for plan III. An optimal sampling distance would be approximately 2.5 m.

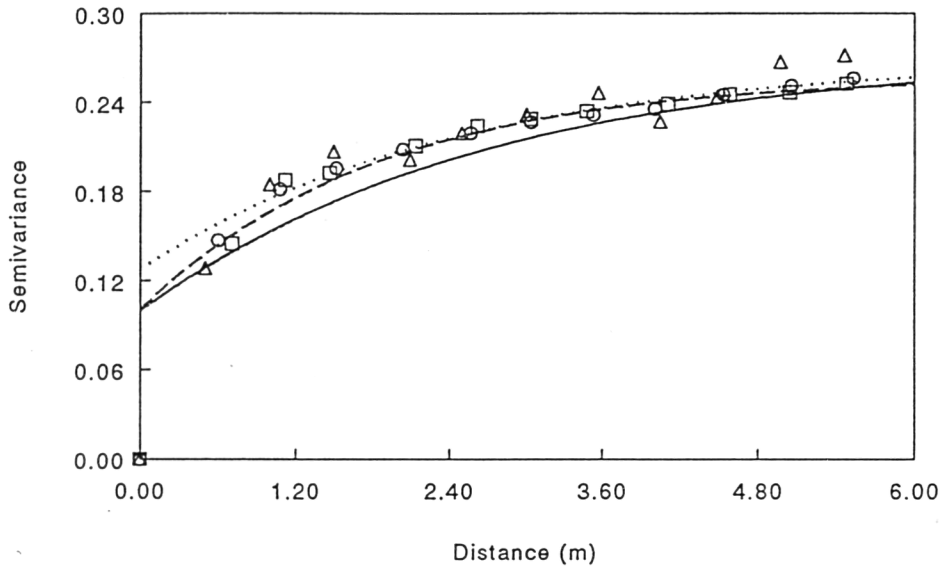

Fig. 3 Semivariograms of black rot disease incidence for sampling plan I (O ———; scoring all the 1600 plants), II (□ ·····; scoring one out of every three plants), and III (△ – – – –; scoring one out of every five plants).

Directional semivariograms were calculated in northeast and southeast direction, to search for anisotropy resulting from predominant south-western winds during rainfall. Anisotropy was indeed found (Fig. 4). The semivariogram based on north-east distances had an eight times greater range (8.9 m) than the south east semivariogram (1.1 m). This result confirms the assumption that disease was spread with splashing rain storms blowing predominantly from the south west.

Disease incidence estimates for the three sampling plans were quite similar: 45.6, 43.1 and 44.9% for plans I-III, respectively. Kriging for plans II and III reproduced the observed disease incidence pattern accurately (Fig. 5). In this figure, each square represents the disease incidence per 4 plants. The distribution of black rot was not

homogenous; incidence was higher in the centre of the plot. Kriging based on plan II reproduced this pattern well; kriging based on plan III reproduced it less well, but the representation of the actual pattern is still acceptable.

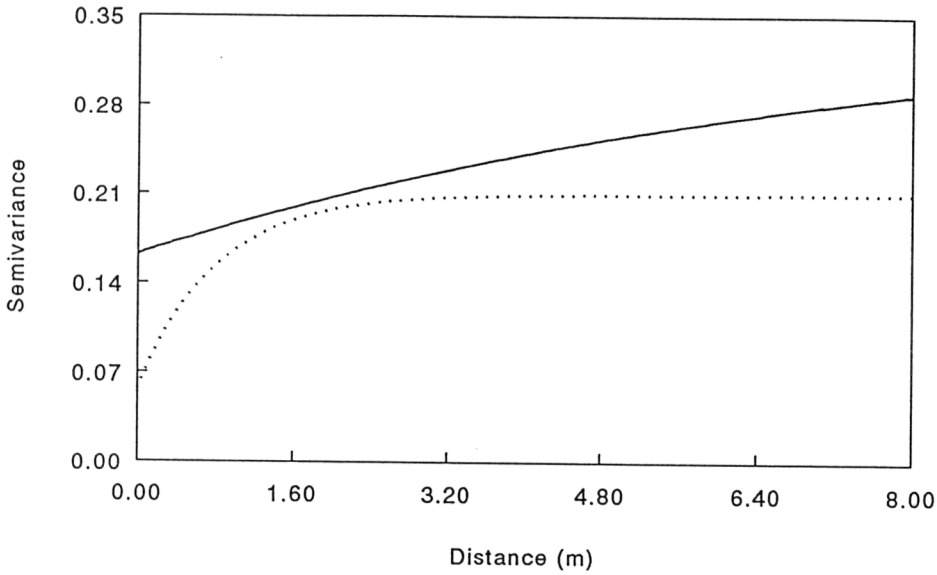

Fig. 4 Directional semivariograms of black rot disease incidence for sampling plan I. South west (prevailing wind): ———; North west (perpendicular to the wind): ----------

Fig. 5 Actual pattern of black rot incidence on 24 July 1990 and kriging maps based on sampling intensities of 33% (plan II) and 20% (plan III). Each square represents a quadrate of 2 x 2 plants. The intensity of shading indicates incidence in these quadrates, running from 0/4 infected plants (blank) to 4/4 infected plants (black).

This example illustrates the usefulness of geostatistics for analyzing and mapping spatial patterns. It was possible to reduce the number of samples with 80% and still obtain sufficient information about the spatial pattern. Sampling effort could probably not have been further reduced than this because the range of influence in the semivariogram was 2.5 m. When spatial correlation extends further, greater savings in sampling effort are attainable. For instance, Lecoustre *et al.* (1989) found that a sample size of 7% of all plants sufficed to assess the spatial pattern of African cassava mosaic virus.

Case: developing and evaluating protocols for mite monitoring

Fruit tree red spider mite, *Panonychus ulmi*, is a potential pest in apples worldwide. It can be controlled naturally by predators, but biological control is easily upset by pesticides. In apple crops in the state of New York, monitoring from early June to late August is required to make sure that biological control is effective. Schemes for efficient monitoring over time in this system were developed and evaluated by Nyrop & van der Werf (1994) and Nyrop *et al.*, (1994). The approach can be readily transferred to other systems.

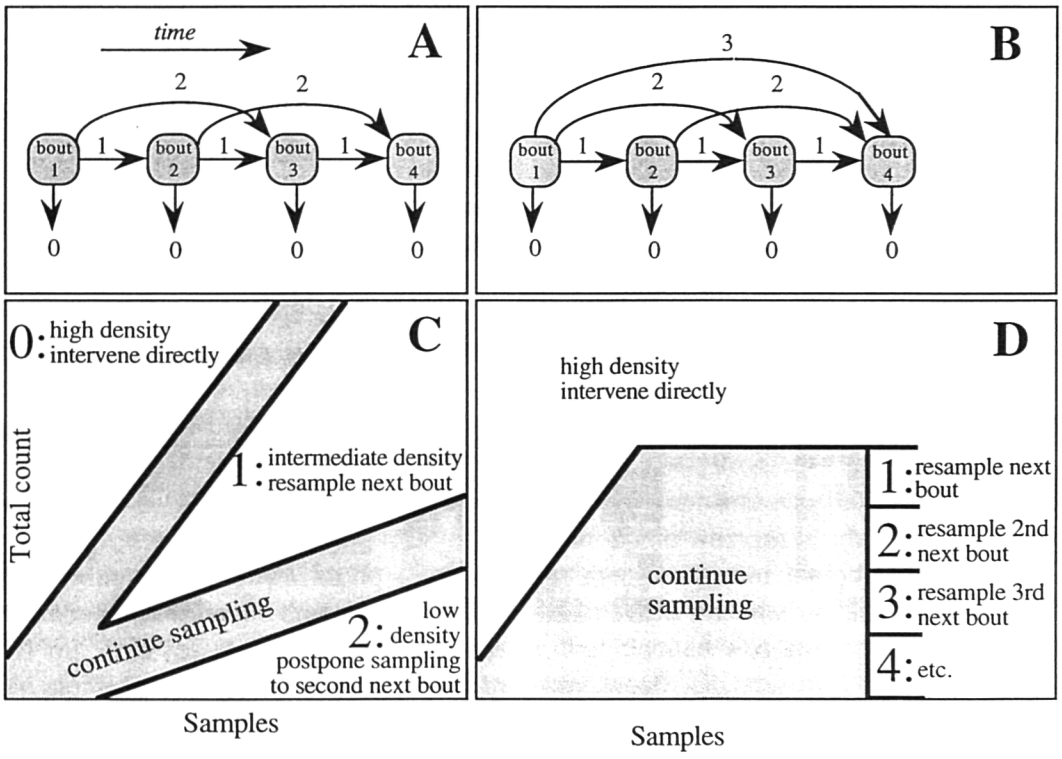

Fig. 6 Mite monitoring protocols (A & B) and the constituent sequential sampling plans (C & D). For explanation see text.

One method (cascaded tripartite sequential classification; TSC; Fig. 6A) was constructed by serially combining in time sampling plans that classify density into one of three categories with according management consequences 0 (intervene), 1 (sample at next occasion), and 2 (sample at second next occasion; Fig. 6C). The other protocol (adaptive frequency classification; AFC; Fig. 6B) was constructed by cascading in time sampling plans that are based on a combination of sequential classification and estimation of density (Fig. 6D). AFC allows sampling to be postponed more than two periods when density is unlikely to grow to damaging levels within that time.

In both schemes, timing and frequency of sampling are adjusted to the demands and possibilities of the actual situation, as indicated by sampling observations and a prediction of dynamics. The most suitable parameters for both schemes were found by simulating monitoring performance for fictitious and historical pest population trajectories. According to simulation, both methods scheduled interventions at appropriate times. The simulation results for the monitoring based on tripartite sequential classification were confirmed in a field evaluation involving 42 orchard blocks. Both methods use fewer sampling resources than sampling at pre-defined times, which is the usual method in practice. AFC-based plans required less sampling than TSC-based plans. Simulation further indicated that the currently used action threshold for red mites in the North Eastern USA are too low, resulting in spray recommendations when there would still be opportunity for natural control by predatory mites. TSC and AFC provide a framework for objectively evaluating and optimizing monitoring protocols for a range of pests and diseases.

Case: developing a detection method for nematode patches

Potato cyst nematodes (PCN) are not indigenous to Europe. They were introduced together with the potato plant from Central and South America. The presence of PCN manifested itself only in the 20th century when potatoes were grown in narrower rotations. Fields are free of PCN until an initial introduction occurs, mostly by seed potatoes. After introduction the nematode multiplies every year in which a host is grown. Active mobility of the nematode occurs after egg hatch, when the juveniles search for root tips to penetrate. This active movement would result in a dispersal of only a few centimeters per year. After maturing, the new generation of PCN overwinter as eggs inside the hardened dead body (cyst) of the female. Therefore, PCN are concentrated at locations where plant have grown. Horizontal and vertical redistribution of nematodes in the soil depends upon farming practices as soil tillage and harvesting. Dispersal from field to field occurs by pure chance when clumps of soil with cysts adhere to agricultural machinery or harvested potatoes.

In the newly reclaimed polder areas of the Netherlands, PCN infestations are young. They occur in the form of distinct patches in otherwise uninfested fields. Detection

methods for these PCN patches are being used in legislation, quarantine, certification of potatoes destined for export, and (most important) for guiding nematode control, e.g. by growing resistant cultivars. There was a need for estimating the error rates of the detection methods and to optimize (if possible) these methods.

As a first step, the shape of nematode patches was studied. About 40 farmer's fields, which, according to the statutory soil sampling protocol, were regarded as PCN infested, were sampled twice. The first sample was used to locate the infestation focus. The second sample was aimed at accurately mapping the spatial distribution of the cysts in the focus. Soil samples of at least 1.5 kg per m^2 were collected and processed.

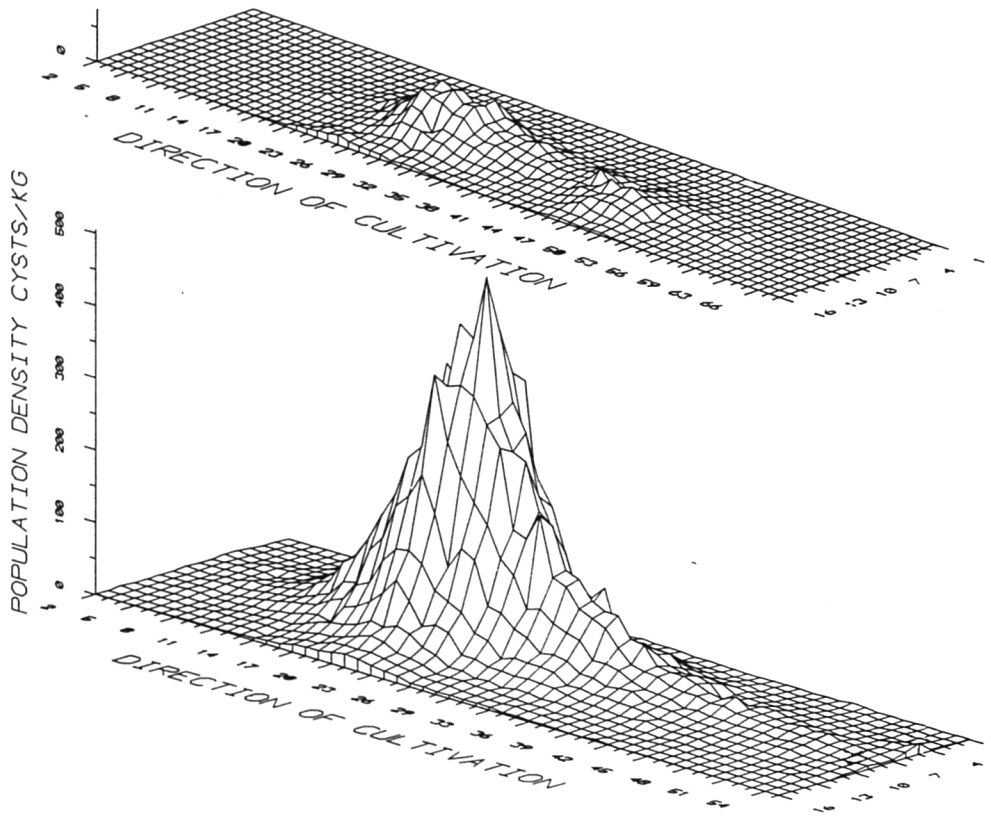

Fig. 7 Two foci of potato cyst nematode on heavy marine clay soil in a recently infested area. Above: small focus with a population density of 85 cysts per kilogram soil in the center of the focus. Below: large focus with more than 500 cysts per kilogram soil in the center of the focus. Both foci were mapped by sampling each square meter and collecting 2.5 kg of soil per m^2.

All foci were more or less elliptical with the largest population densities in the center. From this point the densities decreased exponentially. The decrease was slower in directions parallel to the rows than across them (Fig. 7), i.e. the patches had the greatest extension along the path of the machinery. The spatial extension of foci was also greater in the driving direction than in the reverse direction. (Because machinery has standard width, farmers may year after year follow the same driving pattern and directions over the field.)

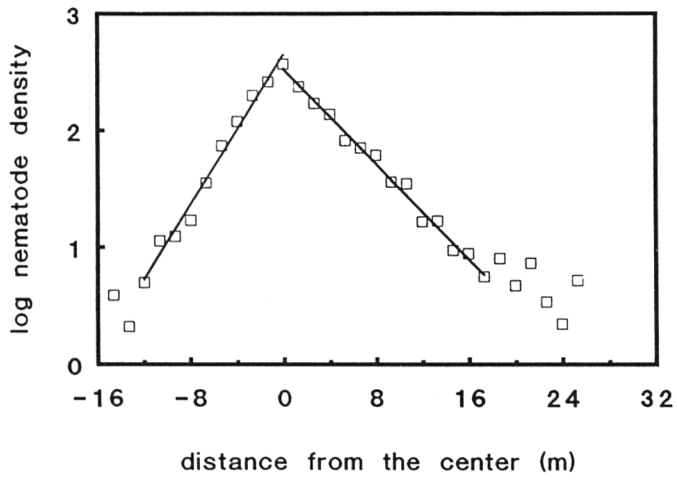

Fig. 8 Linear relationship between logarithm of nematode density and distance from the center of the focus. Squares represent actual cyst counts in samples of 2.5 kg soil, originating from the middle row of successive square meter plots in the direction of cultivation of the large focus depicted in Fig. 7. Samples with fewer than 5 cysts per kilogram soil were omitted when fitting the drawn regression line.

A linear relation between log population density and the distance from the center of a focus (Fig. 8) was found and parametrized (Schomaker & Been, 1992):

$$\mathrm{E}\left(N_{x,y}\right) = N_{0,0}\, L^{x}\, B^{y}$$

where

$\mathrm{E}\left(N_{x,y}\right)$	is the expected density at location (x,y)
(x,y)	is the location relative to the centre of the focus, with x measured in the direction of cultivation and y measured across
$N_{0,0}$	is the density in the centre of the focus
L	is the fractional decrease of expected density per meter departure from the centre along the rows, i.e. in the x direction

B is the fractional decrease of expected density per meter departure from the centre across the rows, i.e. in the y direction.

The frequency distributions of L and B in the 40 fields were approximately normal.

The frequency distribution of numbers of cysts in 1.5 kg samples from small areas as used for mapping the foci, is adequately described by a negative binomial distribution with a value of 70 for the parameter k. The probability of finding no cysts in a sample is then given by the zero-term of the negative binomial distribution.

An infestation focus is detected if one or more cysts are extracted from it. The detection probability of a focus can therefore be defined as 1 minus the probability that cysts were found in none of the subsamples taken from the focus. In the Netherlands and in most other countries sampling according to a rectangular grid pattern is customary. The distance from one core to the next in both directions determines how many subsamples are taken from a certain area. Sampling grid and auger size determine the total amount of soil collected. A sampling grid can be superimposed on a focus in many ways. Each overlay pattern of focus and sampling grid results in a different detection probability. A computer program was written to calculate the detection probability when shifting the sampling grid longitudinally and laterally, relative to the focus.

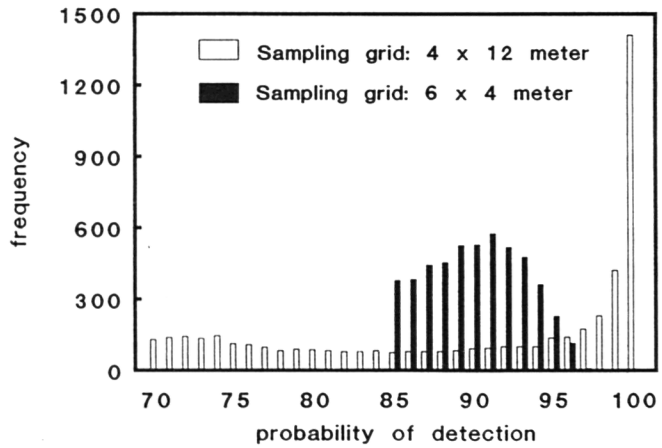

Fig. 9 A comparison of the frequency distributions of detection probability using a 6 x 4 m and a 4 x 12 m sampling grid (length x breadth). The core size was optimized to obtain an average detection probability of 90% for a nematode focus with 50 cysts per kilogram soil in the center.

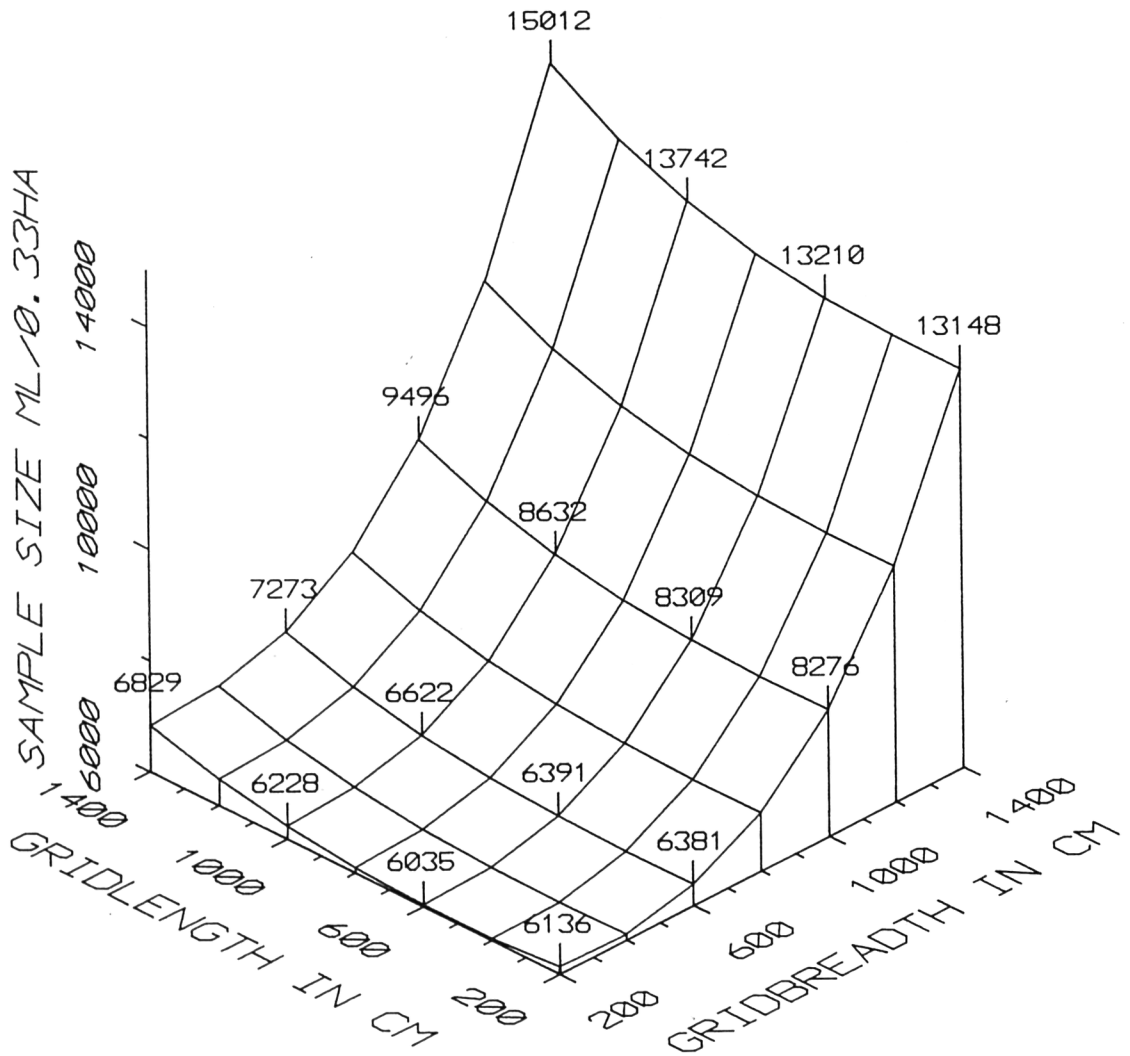

Fig. 10 A comparison of the sample size per 0.33 ha when the sampling grid was varied between 2 x 2 m and 14 x 14 m. For each grid, the core size were determined that yielded a 90% chance of detecting a single focus with 50 cysts per kg soil in the center. The total sample size is the product of core size and the total number of samples as determined by the grid.

Fig. 9 shows the frequency distributions of detection probabilities of two different sampling grids. The program uses the exponential equation that describes the spatial profile of foci to calculate the expected population densities throughout a predefined focus. The probability of detecting no cysts at a certain location in the focus was calculated with the negative binomial distribution. As detection with a low failure rate is required, the parameters L and B were set to relatively small values (10% percentiles of the observed distributions of L and B), reflecting steep gradients. This

'worst case approach' ensures that for 90% of the patches, the specified detection probability is actually attained. For 10% of the patches (the steepest ones), the detection chance will be lower than specified, and the overall detection chance for the whole population of patches will be better than specified. Calculations for a given sampling grid were made of the amount of soil per core and in total that had to be collected for a 90% detection probability of the predefined focus.

In Fig. 10, the required total sample sizes (g soil) per one third of a hectare are compared, when using different sampling grids. The narrowest grid had sampling intervals of 2 x 2 m; the widest grid 14 x 14 m. Iterations were made for sample size until, with each grid, a focus of 50 cysts per kg soil in the center was detected with 90% probability. The optimal sampling grid (in terms of the required amount of soil) was the 6 x 4 m grid (length x breadth), which required a total sample of 6 kg soil. To obtain the same 90% detection chance with a 4 x 12 m grid, 11 kg soil had to be collected and analysed.

Potato cropping frequencies differ among growing areas in the Netherlands, and among European countries. It is likely that the differences in cropping practices are reflected in spatial patterns of PCN, which affects the performance of sampling patterns. The required detection probability depends upon the product. Seed potato growers, always alert with regard to export requirements, require more precise detection methods than consumption potato growers. As a result of differences in spatial patterns and required detection probability, tailor-made sampling methods are desirable. The presented approach allows the design of sampling methods that are tailor-made for the respective target areas and product groups.

EPILOGUE

This paper draws together some techniques that are used in the study of plant pest and diseases, and could be profitable in the young research field of entomopathogenic nematodes. Progress in ecological research on EPNs is presently hampered by technical difficulties in retrieving nematodes from soil and by lack of knowledge on spatial patterns and sampling distributions. For entomopathogenic nematodes, most of the basic work on spatial patterns and sampling distributions that is necessary for the design of efficient sampling plans has still to be done. It is hoped that this presentation of research on pests and diseases provides ideas and stimulus to undertake such work and provide a sound basis for further ecological work.

The selection of techniques in this paper is necessarily restricted. A comprehensive treatise of techniques for spatial statistical analysis is given by Upton and Fingleton (1985; 1989). No mention is made here of techniques for modelling spatial *processes*. Such spatial modelling may explain spatial phenomena in relation to the

causal relationships and processes, contrary to statistical analyses, that can only describe and quantify correlations and trends, but cannot explain them. Overviews of approaches to spatial modelling are given by van der Werf *et al.* (1989) and Holmes *et al.* (1994). A potentially relevant new development are techniques of precision farming or site-specific management. These techniques are targeted at providing the appropriate management action to each site in a field, e.g. in response to soil fertility level (Wollenhaupt *et al.*, 1994; Bouma *et al.*, 1995) or weed development (Christensen *et al.*, 1994).

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REFERENCES

Binns, M.R., 1994. Sequential sampling for classifying pest status. In: L.P. Pedigo & G.D. Buntin: Handbook of sampling methods for arthropods in agriculture. CRC Press, Boca Raton, p. 138-173.

Bouma, J., J. Brouwer, A. Verhagen & H.W.G. Booltink, 1995. Site specific land management on a field level: high and low tech approaches. In: Conference Proceedings on Eco-regional Approaches for Sustainable Land Use and Food Production, Den Haag, Netherlands, 12-16 December 1994.

Burrough, P.A. 1987. Spatial aspects of ecological data. In: R.H.G. Jongman, C.J.F. ter Braak & O.F.R. van Tongeren (Eds): Data analysis in community and landscape ecology. Pudoc, Wageningen, p. 213-251.

Chellemi, D.O., K.G. Rohrbach, R.S. Yost & R.M. Sonoda, 1988. Analysis of the spatial pattern of plant pathogens and diseased plants using geostatistics. Phytopathology 78, 221-226.

Christensen, S., E. Nordbo & K. Kristensen, 1994. Weed cover mapping with spectral reflectance measurements. In: P. Brain, S.H. Hockland, P.D. Lancashire & L.C. Sim (Eds): Sampling to make decisions. Aspects of Applied Biology 37, p. 171-179.

Fowler, G.W. & A.M. Lynch, 1987. Sampling plans in insect pest management based on Wald's sequential probability ratio test. Environmental Entomology 16, 345-354.

Holmes, E.E., M.A. Lewis, J.E. Banks & R.R. Veit, 1994. Partial differential equations in ecology: spatial interactions and population dynamics. Ecology 75, 17-29.

Hudelson, B.D., M.K. Clayton, K.P. Smith, D.I. Rouse & C.D. Upper, 1989. Nonrandom patterns of bacterial brown spot in snap bean row segments. Phytopathology 79, 674-681.

Journel, A.J. & Huijbregts, C.J., 1978. Mining geostatistics. Academic Press, New York, 600 pp.

Lannou, C. & S. Savary, 1991. The spatial structure of spontaneous epidemics of different diseases in a groundnut plot. Netherlands Journal of Plant Pathology 97, 355-368.

Lecoustre, R., D. Fargette, C. Fauguet & P. Reffye, 1989. Analysis and mapping of the spatial spread of African cassava mosaic virus using geostatistics and the kriging technique. Phytopathology 79, 913-920.

Munkvold, G.P., J.A. Duthie & J.J. Marois, 1993. Spatial patterns of grapevines with Eutypa dieback in vineyards with or without perithecia. Phytopathology 83, 1440-1448.

Nelson, M.R., R. Felis-Gastelum, T.V. Orum, L.J. Stowel & D.E. Meyers, 1994. Geographical information system and geostatistics in the design and validation of regional plant virus management progress. Phytopathology 84, 898-905.

Nyrop, J.P. & M.R. Binns, 1991. Quantitative methods for designing and analyzing sampling programs for use in pest management.In: D. Pimentel (Ed.): CRC handbook of pest management in agriculture, p. 67-132.

Nyrop, J.P., M.R. Binns, W. van der Werf & J. Kovach, 1994. Cascading tripartite binomial classification plans to monitor European red mite (Acari, Tetranychidae) through a season; development and evaluation of a new methodology for pest monitoring. Experimental & Applied Acarology 18, 123-153.

Nyrop, J.P. & Van der Werf, W., 1994. Sampling to predict or monitor biological control. In L.P. Pedigo and G.D. Buntin (Eds.): Handbook of Sampling Methods for Arthropods Pests in Agriculture. CRC Press, Boca Raton, Florida. p. 245-336.

Stein, C.G. A., Kocks, J.C. Zadoks, H.D. Frinking, M.A. Ruissen & D.E. Myers, 1994. A geostatistical analysis of the spatio-temporal development of downy mildew epidemics in cabbage. Phytopathology 84, 1227-1239.

Stern, V.M., R.F. Smith, R. van den Bosch & K.S. Hagen, 1959. The integration of chemical and biological control of the spotted alfalfa aphid, Part I: the integrated control concept. Hilgardia 29, 81-101.

Schomaker, C.H. & T.H. Been,1992. Sampling strategies for the detection of potato cyst nematodes: developing and evaluating a model. In: F.J. Gommmers & P.W.Th. Maas, (Eds): Nematology from molecule to ecosystem. Proceedings of the Second International Nematology Congress, 11-17 August 1990, Veldhoven, the Netherlands. Dekker & Huisman, Wildervank, the Netherlands. p. 182-194.

Upton, G.J.G. & B. Fingleton, 1989. Spatial data analysis by example. Volume 1. Point pattern and quantitative data. Wiley, Chichester, 410 pp.

Upton, G.J.G. & B. Fingleton, 1989. Spatial data analysis by example. Volume 2. Categorical and directional data. Wiley, Chichester, 416 pp.

van der Werf, W., W.A.H. Rossing, R. Rabbinge, M.D. de Jong & P.J.M. Mols, 1989. Approaches to modelling the spatial dynamics of pests and diseases. In: R. Cavalloro and V. Deluchi (Eds): PARASITIS 88, Proceedings of a scientific

congress, Barcelona, 25-28 October 1988, p. 89-119.
Wilson, L.T., W.L. Sterling, D.R. Rummel & J.E. DeVay, 1989. Quantitative sampling principles in cotton IPM. In: Frisbie, R.E., K.M. El-Zik & L.T. Wilson (Eds): Integrated pest management systems and cotton production. John Wiley & Sons, New York, p. 85-119.
Wollenhaupt, N.C., R.P. Wolkowski & M.K. Clayton, 1994. Nutrient management; mapping soil test phosphorus and potassium for variable-rate fertilizer application. Journal of Production Agriculture 7, 441-448.

SMALL SCALE DISTRIBUTION OF *STEINERNEMA FELTIAE* JUVENILES IN CULTIVATED SOIL

Sergei E. Spiridonov[1] and Dmitrii A. Voronov[2]

[1]Institute of Parasitology, Russian Academy of Science, Leninskii pr. 33, 117071 Moscow, Russia. [2]Institute for Information Transmission Problems, RussianAcademy of Science, Ermolova st. 19, 101447 Moscow, Russia

SUMMARY

The distribution of steinernematid juveniles was studied in a small (2 x 2.5 m) plot of cultivated soil in southern Estonia. Soil samples were collected at 5 cm intervals along eight parallel transects The extraction efficiency of a modified Baermann funnel method was estimated by adding 100 vitally stained juveniles of *Steinernema feltiae* to each sample before processing and was found to be constant though low (7.9%). Juveniles of *S. feltiae* were distributed unevenly along the transects: peaks of several dozens of juveniles were found against a low-level background with only a few juveniles per sample.

INTRODUCTION

Little is known about the distribution and dynamics of the free-living third stage infective juveniles (IJs) of Steinernematidae in natural soil habitats (Hominick, 1990). Such data will probably be important for the understanding of steinernematid natural ecology. Numbers of IJs in soil cores were estimated by their direct extraction.

METHODS

The small-scale distribution of steinernematid IJs was studied in a small, fallow plot (last cultivation in 1989) of sandy soil in southern Estonia in 1990-91. Soil cores of 20 cm length and 3.7 cm diameter (volume 215 cm^2) were taken with an auger. The total number of samples was 393. Samples were taken at 5 cm intervals along parallel transects; each transect contained about 50 samples (seven samples were deleted because of stones and mouse or mole holes). Initially, in August 1990, two transects - Aug90a and Aug90b - were established two metres apart. The following year, an additional six transects were established, two in each of May, June and August. The final pattern consisted of eight transects in two groups: a group of five transects 5 cm apart (in the following order: Aug91b, Jun91b, Aug90a, Jun91a, Aug91a) and, two metres away, a group of three transects, also 5 cm apart (in the following order: May91b, Aug90b, May91a). The resulting pattern was a 5 x 50 sample grid and a 3 x 50 sample grid with 5 cm spacing between adjacent samples.

The soil cores were processed by a slightly modified Baermann extraction method, as follows. Each soil core was dispersed in a beaker of water immediately after extraction. Large pieces of stone were removed by hand. The soil was then rinsed into a tall 1.5 l glass cylinder. The sample was mixed by inverting the cylinder

(sealed with the palm of the hand) five times. Soil particles were allowed to sediment for 30 sec, after which the supernatant was filtered through a 100 μ mesh sieve. This procedure was repeated five times. In the first and second decantation, the supernatant was first poured through a coarse (1 mm) mesh sieve to remove organic debris. The final residue was put on a 300 cm^2 nylon sieve with 100 μ openings for filtration under water for 3-4 hours at 18-24°C. The filtrate was concentrated into a 50 ml beaker, and examined in a counting chamber consisting of a serpentine groove in a plexiglass plate. All extracted rhabditid juveniles were studied for the presence of features characteristic of steinernematid IJs : small bowl-shaped stoma, closed and nonfunctional oesophagus, conical tail and, most important, a bacterial vesicle in the proventricular part of the intestine. The identification of isolated IJs as steinernematids was confirmed following their release on agar plates with *Xenorhabdus* bacterial lawns. Though all of the obtained adult steinernematids were identified as *Steinernema feltiae*, still the possibility that other steinernematid IJs were present in the same soil plot cannot be excluded.

To determine the efficiency of the extraction procedure, one hundred *S. feltiae* IJs isolated from the same soil and vitally stained with Nile Blue (Spiridonov, 1991) were added to each core before processing. Stained IJs were counted in the final residue of the extraction. The mean recovery of stained IJs was determined as 7.9%, and the distribution of their numbers fitted the binomial ("Statgraphics" program was used). Thus, the extraction technique was considered sufficiently constant, and the probability of IJ extraction was the same for all samples.

RESULTS AND DISCUSSION

The distribution of extracted *S. feltiae* IJs in transects Aug90a, Jun91a, Aug91a is presented in Figure 1; in transects Aug90a, Jun91b, Aug91b is presented in Figure 2; and in transects Aug90b, May91a, May91b is presented in Figure 3. In Table 1 for each transect the mean value, the variance, the ratio variance/mean value, and the standard error of this ratio (Greig-Smith, 1964) are presented. Only in transect May91a does the distribution fit the Poisson, as the variance is very close to the

TABLE 1. Statistical properties for each transect

Transect	Aug9a	Jun91a	Aug91a	Jun91b	Aug91b	Aug90b	May91a	May91b
Number of samples	49	52	47	52	52	47	47	47
Mean number of IJs per sample (m)	6.02	6.21	4.64	4.08	7.38	4.62	1.36	2.21
Variance (v)	56.7	171	48.2	26.3	229	42.9	1.59	5.70
v/m	9.41	27.5	10.4	6.44	31.0	9.30	1.17	2.58
St. error of v/m	.204	.198	.209	.198	.198	.209	.209	.209

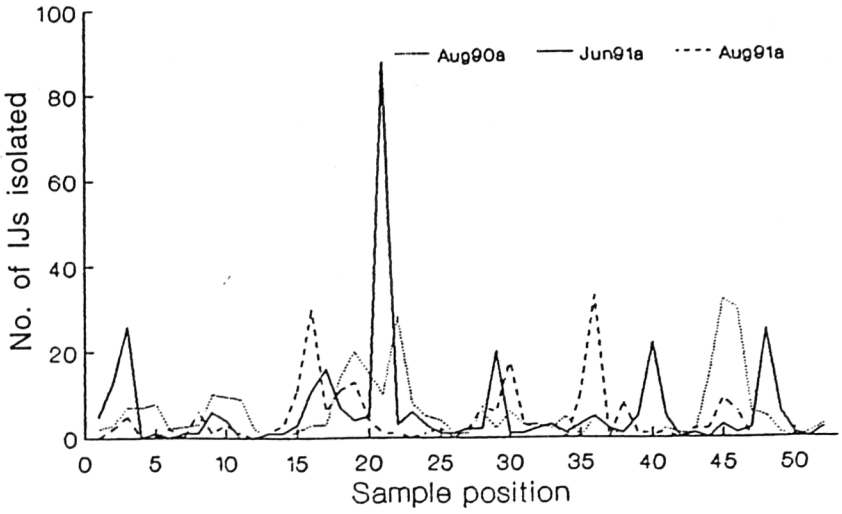

Fig. 1. Infective juvenile (IJ) distribution in three close transects Aug90a, Jun91a and Aug91a

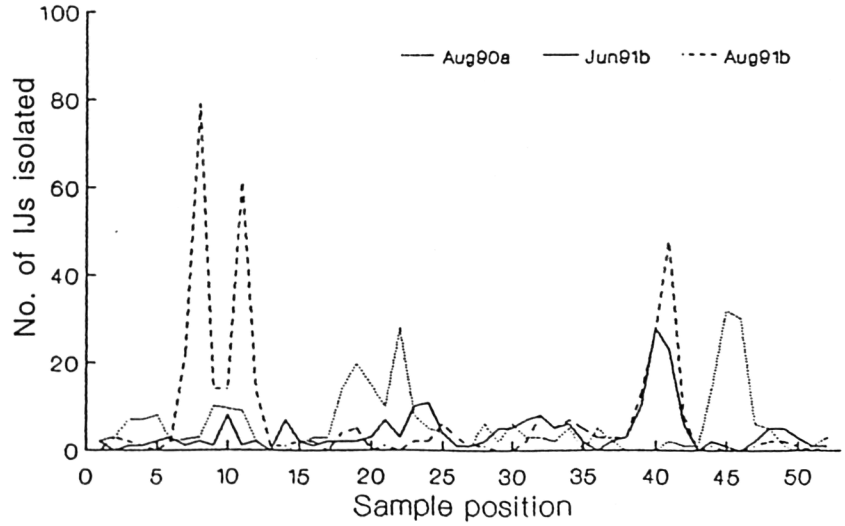

Fig. 2. Infective juvenile (IJ) distribution in three close transects Aug90a, Jun91b and Aug91b

mean value (Table 1). In all other transects it is contagious (aggregated), because the variance significantly exceeds the mean value. Such a distribution of IJs in these transects could not have resulted from the low extraction efficiency, because it is known that the loss with a constant probability of objects from studied samples cannot transform a primarily regular or Poisson distribution into a contagious distribution (Feller, 1970). The majority of samples contained only small numbers of IJs; in addition, however, samples with an abundance of IJs can be found. The autocorrelation, or autocorrelation coefficient (Yule and Kendall, 1950; Handbook of applicable mathematics, 1984) within each transect quickly decreased with an increase of shift - i.e. when the profile reflecting IJ numbers in the transect was moved on 1, 2, 3, etc. steps (Fig. 4). When the shift is equal to 0, autocorrelation by definition is equal to 1; when the shift is equal to 3 or 4, it is on average equal to 0. Consequently, 15-20 cm is the characteristic dimension of IJ peaks. Remarkably, in the transect May91a (solid line in Fig. 4) the autocorrelation is approximately equal to 0 when the shift of autocorrelation is equal to 1, providing additional evidence that in this transect the distribution really fits the Poisson.

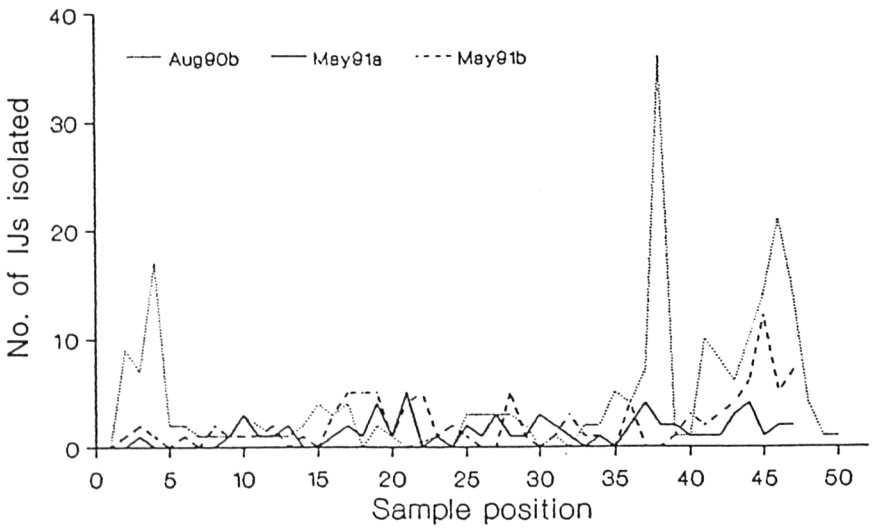

Fig. 3. Infective juvenile (IJ) distribution in three close transects Aug90b, May 91a and May91b

Taking into account the results of the autocorrelation analysis, it is not surprising that the correlation between transects was very low. The correlation coefficient between pairs of neighbouring transects was: Aug90a/Jun91a 0.14, Aug90a/Jun91b -0.21, Jun91a/ Aug91a 0.00, Jun91b/Aug91b 0.29, Aug90b/May91a 0.09, Aug90b/ May91b 0.22. Probably, a complex of spatial and

temporal factors obscured all possible correlation between samples in neighbouring transects.

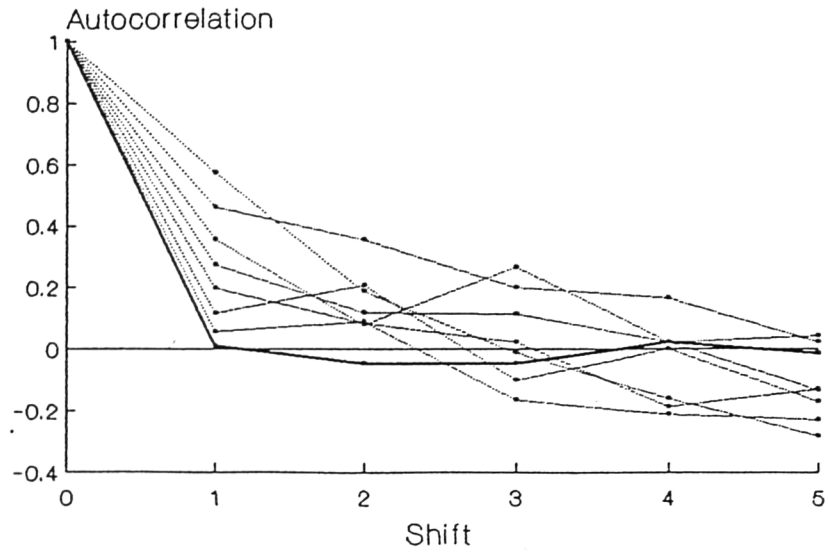

Fig. 4. Autocorrelation coefficient for all eight transects

In general, the pattern of IJ field distribution can be considered as a composition of Poisson low-level background with separate narrow peaks. To prove this statement, for each transect (excluding transect May91a which fitted the Poisson) samples with high numbers of IJs were sequentially excluded until the distribution of the remaining samples fitted the Poisson. The exclusion of samples with abundant IJs was stopped when the variance/mean ratio for the altered transect did not exceed 1 plus 2 standard errors. For example, after excluding samples with more than 7 IJs from the transect Aug90a the remaining 38 samples fitted a Poisson distribution with mean value 2.76 IJs per sample. Analogously, for the other transects the results were (the order of numbers is: maximal value of IJs per sample, number of samples and the mean number of IJs per sample): Jun91a - 7, 44, 2.32; Aug91a - 6, 36, 1.72; Jun91b - 7, 45, 2.53; Aug91b - 7, 41, 1.83; Aug90b - 7, 39, 2.21; May91b - 5, 42, 1.64. For all transects, the number of samples remaining when those from peaks were eliminated was equal to 332, or 84% of the total number of samples.

Analyzing the IJ distribution, we can presume that the peaks of nematode population represent the results of singular acts of recent insect infestation, while the low-level background is occupied by the "old" IJs. This hypothesis is supported by their observed appearance in samples with numerous and only few IJs, which have plenty of lipid granules in the gut of the former, but usually depleted reserves in the latter.

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REFERENCES

Feller, W. (1970). *An introduction to probability theory and its applications.* Vol. I. 3rd ed. John Wiley & Sons, NY.

Greig-Smith, P. (1964). *Quantitative plant ecology.* 2nd ed. Butterworths, London.

Handbook of applicable mathematics. (1984). Vol. VI: Statistics, Part B. Ed. by E. Lloyd. John Wiley & Sons, Chichester.

Hominick, W.M. (1990). Entomopathogenic rhabditid nematodes and pest control. Parasitol. Today **6**, 148-152.

Spiridonov, S.E. (1991). Vital staining of invasive larvae of Steinernematidae (Nematoda, Rhabditida). Angew. Parasitol. **32**, 55-56.

Yule, G.U. and Kendall, M.G. (1950). *An introduction to the theory of statistics.* 14th ed. Ch. Griffin & Co, Ltd, London.

CONCEPTS AND PROSPECTS FOR MODELLING THE EFFICACY AND ECOLOGY OF ENTOMOPATHOGENIC NEMATODES

Wopke van der Werf[1], Peter H. Smits[2], Martin Downes[3] & Christine Griffin[3]

[1]Wageningen Agricultural University, Department of Theoretical Production Ecology, P.O. Box 430, 6700 AK Wageningen, The Netherlands. [2]DLO Research Institute for Plant Protection, P.O. Box 9060, 6700 GW Wageningen, The Netherlands. [3]Saint Patrick's College, Department of Biology, Maynooth, Co. Kildare, Ireland

SUMMARY

Simulation models can improve insight in the ecology of entomopathogenic nematodes (EPNs) and their potential as biocontrol agents of soil pests. Models that include environmental and behavioural factors that are critical for the efficacy of an inundative release, may be used for guiding application strategies, EPN biocontrol-product formulation and genetic engineering of EPNs. Multi-generation population dynamical model provide a tool for estimating persistence, thus allowing assessment of risks associated with releasing genetically modified EPNs into the environment. Constructing a simulation model for one or more species of entomopathogenic nematode requires the retrieval and integration of existing knowledge of the quantitative ecology and behaviour of EPNs. Additional process-oriented research must be carried out to fill gaps in the available knowledge. The validation of simulation models will involve experiments under field conditions.

INTRODUCTION: MODELLING APPROACHES IN ECOLOGY AND CROP PROTECTION

A model is a simplified representation of a system and a system is a limited part of reality. Mathematical models represent numerical relationships between elements of a system. There are many different types of mathematical models and many criteria to classify them, e.g. process-based *versus* statistical, dynamic *versus* static, deterministic *versus* stochastic, and spatially explicit *versus* temporal (Peters, 1991; De Wit, 1993; Hurd & Kaneene, 1993). The character of a model depends foremost on its purpose. In crop protection ecology, three categories of models are prevalent: analytical models, simulation models, and descriptive models. These models differ in many aspects, including the level of aggregation and simplification, structure, purpose, methodology and data requirements (Table 1). These three modelling approaches could be characterized as speculative, mechanistic and correlative.

Table 1: Characterization of analytical models, simulation models and descriptive models in crop protection ecology

	Analytical models	Simulation models	Descriptive models
Characteristic	dynamic one to few equations	dynamic many equations	seldom dynamic one to few equations
Level of aggregation and simplification	high	low elaborate biological detail hierarchical: two levels, system & process level	high or intermediate
Purpose	abstraction generalization insight in principles	relationship processes <-> system influence environment insight in specifics	prediction
Examples	exponential & logistic growth predator-prey models Janssen & Sabelis (1992)	SeMNPV (see text); Nachman (1991) van den Bos & Rabbinge (1976); van der Werf et al. (1989)	Yellowing viruses, cereal diseases (see text)
Methodology	mathematical analysis: analytical integration stability analysis of equilibria	simulation of dynamics numerical integration sensitivity analysis simplification into decision rules	statistical regression
Required skills	mathematics	experimentation & programming	data survey and statistics
Data requirements	often loose relationship to data	detailed knowledge of life cycle (process level) data for model validation (system level)	elaborate data set
Disadvantages	oversimplification, irrealistic difficult to understand for biologists	lacking data laborious to verify code lengthy documentation difficult to keep overview	little or no insight little generality

Analytical models summarize the main components of dynamic biological systems in a few equations that characterize the rates of change of the state variables. The foremost aim of analytical models is to study general principles underlying dynamic systems behaviour. Analytical models characterize a whole class of systems and their predictions, formulated as general insights, have wide validity. Such predictions may be difficult to operationalize in a specific system. An example of an analytical model of interacting pest and enemy populations is the system of differential equations

$$\begin{cases} \frac{dx}{dt} = \alpha x - \beta y \\ \frac{dy}{dt} = \gamma y \end{cases}$$

where x is the state variable prey density and dx/dt is its rate of change
y is predator density
α is the relative growth rate of the prey population (assuming unlimited resources)
β is the prey consumption rate per predator (assumed to be independent of prey density)
γ is the relative growth rate of the predator population (assuming unlimited food)

This simple set of equations characterizes some fundamental aspects of the interaction between spider mites and predatory mites in local patches (Janssen & Sabelis, 1992). Analytical integration of the differential equations yields general and testable predictions about the future course of the dynamics of the system. For example, it can be shown that the prey will finally be eradicated if the initial predator/prey ratio is greater than

$$\frac{\alpha - \gamma}{\beta}$$

The equation shows how the critical initial predator/prey ratio is affected by the relative growth rates of the prey and predator populations and by the feeding rate of the predator. These parameters are - of course - dependent upon conditions, such as temperature and host plant quality. This model serves the purpose of providing insight quite well.

The assumption of a constant consumption rate, independent of prey density, is only tenable if prey density is high enough. Inclusion of a curvilinear relationship between prey density and predator feeding rate would make the model more truthful. If that is done, analytical solution of the rate equations is no longer possible and the dynamics must be investigated by simulation. By making more realistic assumptions about the system, the model gradually develops into a simulation model.

Analytical models are criticized - and often rightly so - by biologists for being oversimplified, which makes their results less credible. Moreover, the mathematics involved in many papers on analytical models deters interest by biologists, especially if the results of mathematical analysis are not confronted with biologically interesting questions. Nevertheless, analytical models are a powerful tool for analysing and

demonstrating general principles in biological systems. Hudson & Norman (this volume) give an example of the application of analytical modelling to EPNs.

Simulation models are complementary to analytical models. They are much less aggregated than analytical models. This means that details of the life cycle, such as stage structure and spatial processes, are often explicitly represented in computer code. The model integrates the processes into a 'grand picture' of the whole system. Such models enable the study of the relationship between individual traits, environmental factors and the behaviour of the system. Simulation models are system specific. Predictions of the model, which are mostly formulated quantitatively, are therefore not of general validity.

An example of such a simulation model is the model for the epidemiology of the *Spodoptera exigua* nuclear polyhedrosis virus (SeMNPV) in a population of beet armyworm (*Spodoptera exigua*) in glasshouse chrysanthemums (de Moed *et al.*, 1990; van der Werf *et al.*, 1991). The model is constructed according to the state variable approach (Leffelaar, 1993).

In the model there is a bookkeeping of the initiation, development and demise of insect-infested crop patches. These patches are classified according to time of initiation and the presence or absence of virus. State variables that characterize patches include the numbers of caterpillars in each stage, the leaf area index of the crop and the density profile of virus over the height of the canopy. Processes within a patch that are modeled include the decay of virus, caterpillar infection by sprayed virus, insect feeding, spatial dispersal of caterpillars within and between plants, encounters between healthy caterpillars and virus-contaminated leaves, the development of caterpillars from one stage to the next, and the development of disease in infected specimens. The insect life-cycle is closed when eggs are laid by adult moths that emerge from the patches, starting a new generation of patches. Depending upon processes within the patch, and subject to stochastic influences, newly laid eggs may be infected or not. Infected eggs close the life-cycle of virus.

An important limitation to simulation models, based on state variables, is the often lengthy code and the difficulty of maintaining a firm conceptual hold of the interrelationships between parts and the whole. Good programming practice is an important tool to secure such overview and to ensure computational correctness, as is mathematical analysis of simplified model versions and limiting cases. Because simulation models incorporate biological details, they invite permanent updating as new insights and data become available. Such updating may or may not be appropriate, depending upon the model's objectives (which is seldom completeness) and the consequences of new insights and data for model behaviour. When making a simulation model it often becomes obvious that data, that are crucial to the model building, are unavailable. Such identification of knowledge gaps is useful for the progress of research and prioritization of research efforts, but the lacks of knowledge may frustrate the timely development and fruitful use of simulation models.

Three phases may be distinguished during the construction of a simulation model and within those phases there are several steps (Rabbinge & de Wit, 1989).

First, there is the *conceptual phase.* It includes at least three steps:

1. Formulation of the objectives
2. Definition of the limits of the system
3. Conceptualization of the system; determining the level of aggregation, simplification and detail, choice of state variables, relationships and external influences.

The conceptual phase results in a conceptual model and work plan for collecting the required process data and working out the model in computer code. The second phase includes at least three steps:

4. Quantification of relationships in the model using literature data and new experiments at the process level
5. Writing the computer code; documentation of the model
6. Testing the components of the model, verification of the code and checking that the performance of the simulation program is in agreement with the input data

The second phase results in a functioning computer program that is in accordance with the knowledge about the system and that is usable for the original (or adapted) objectives. During the second phase a technical documentation of the model and its inputs and outputs must be written. The third phase focuses on further analysis, application and (where possible) simplification of the model.

7. Validation of the model and parts of it, using independent experiments on the system level.
8. Structural and numerical sensitivity analysis
9. Simplification; development of a summary model; scenario studies with the model
10. Formulation of decision rules or forecasting tools to be used in management.

The SeNPV model was conceptualized by the use of state variables which quantify the number or density of individuals or 'amounts' in a certain category. An alternative approach in population models is to represent the individuals themselves and build an individual-based model (IBM; de Angelis & Gross, 1992; van der Werf *et al.*, 1989). This approach is especially appropriate for systems with small numbers of moving individuals in which spatial interactions and chance processes (encounters) are of prime importance.

Individual-based models hold promise for investigating host finding behaviour and movement of EPNs in the soil. The result of an individual based model can be summarized in a functional response formula (Fransz, 1974; Mols, 1993; van Roermund & van Lenteren, 1993), which, in its turn, can be implemented in a simulation model that is based on state variables. When simulating the whole soil-plant-insect-EPN system, computer-time of an IBM is likely to become limiting as EPNs are sprayed in high densities, up to 10^9 individuals m^{-2}.

Descriptive models are calculation tools, that are based on a statistical analysis of data, without an attempt to unravel the underlying mechanisms. They are complementary to analytical and simulation models. Their purpose is to predict an 'output' variable on the basis of knowledge of one or more 'input' variables.

Most descriptive models are static. Examples of this are regression equations that predict disease intensity on a regional scale, based on preceding weather. To predict

mildew severity in winter wheat in the Netherlands, Daamen *et al.* (1992) give the equation

$$y = -132 + 12\,x_1 + 10\,x_2$$

Here, y is predicted percentage of mildew-infested fields, x_1 is the average temperature in the preceding month of October (°C) and x_2 is the average temperature over the period december-March.

To predict the severity of sugarbeet yellows disease in the eastern beet growing area of England, Harrington *et al.* (1989) give the equation

$$y = 111 + 0.20\,x_1 - 68\,x_2$$

Here, y is predicted percentage of virus-infected plants in August, x_1 is virus incidence in the preceding year, and x_2 is the logarithm of the number of days with ground frost in January and February. In the equation, arcsine-transformed virus incidences are used (Snedecor & Cochran, 1989). Shortly before crop emergence, when the virus transmitting aphids *Myzus persicae* have begun to fly, the equation is modified into

$$y = 306 + 0.37\,x_1 - 26\,x_2 - 3.1\,x_3 + 0.0092\,x_3{}^2$$

Here, x_3 is the day (counting from 1 January as day 1) when the first *M. persicae* are caught in a suction trap for aphids in the centre of the eastern sugar beet growing area, near Bury St Edmunds.

The above static regression models are based on biological and empirical insight in what are the key factors in the system and on a thorough statistical analysis of the data set.

Berryman (1991) advocates the use of delayed discrete logistic equations to describe time-series of forest insects. These equations can be used to forecast outbreak years of these insects. Here again, the approach is correlative, but the model is dynamic.

Cellular automata models A class of models that are highly abstract and simplified representations of reality (like the analytical models) are the so-called cellular automata models (Sigmund, 1993). Here, individuals move and perform life functions on a chequerboard, representing the living space. Interactions occur between individuals in adjacent or nearby cells. Offspring is often produced into neighbouring cells. The rules regulating individual behaviour are generally stochastic (involving chance) rather than deterministic. Cellular automata models provide a useful vehicle for exploring the behaviour of spatially distributed population systems (insight function). They lack the specificity and biological realism required for prediction in practical situations. Their role in research is exploratory, like analytical models.

CONCEPTUAL STRUCTURE OF A SIMULATION MODEL FOR ENTOMOPATHOGENIC NEMATODES

Simulation models offer great potential for answering two critical questions with regard to entomopathogenic nematodes. 1. What are the critical factors determining the biological control success of applied entomopathogenic nematodes and how can the BC success be raised by more appropriate application techniques, formulation, environmental circumstances or genetic modification of EPNs?' 2. Will genetically-modified EPNs persist in the environment and how quickly will they or their genes disperse or disappear?

The first question can be answered with a monocyclic model, spanning a single life-cycle of the EPN. The investigation of EPN persistence in a given system requires insight into a series of such lifecycles. This requires a polycyclic model.

In the following a preliminary conceptual model, which is suitable for addressing the first question, is outlined. It bears resemblance to an existing model for plant pathogenic nematodes (van der Werf *et al.*, 1986). The system is a column of soil, surface area 1 m^2, containing spatially distributed insect hosts. The column is subdivided in layers to keep track of vertical redistribution of nematodes (Fig. 1). Vertical redistribution encompasses three processes: 1. mass movement with water fluxes, 2. random movement (diffusion), and 3. directed movement to hosts. No horizontal distribution of nematodes or hosts is taken into account. The frequency of encounters between nematodes and hosts is determined by 1. densities, 2. activity, 3. detection distance, 4. spatial clumping, 5. movement pattern of nematodes.

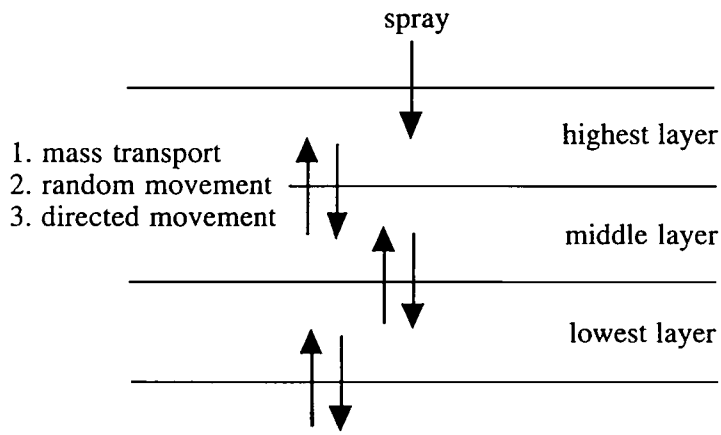

Fig. 1. Vertical movements of nematodes in a layered soil system, to be taken into account in a mono-cyclic model simulating biocontrol by applied EPNs

Studies on insect host finding behaviour provide good examples of how the encounter process may be modeled (Sabelis, 1981; Mols, 1993). Nematodes are subject to mortality and loss of vitality (quality), due to aging and biotic factors (Fig. 2). Infection success depends on vitality. Infected hosts produce a new generation of infective

juveniles. Rates of movement, loss of vitality and infection are dependent on environmental factors, primarily temperature and water availability. Soil texture and structure affects the relationship between water content and suction force (pF). Water relationships affect nematode movement and oxygen availability (Wallace, 1963). The modelling concept is straightforward. Critical steps include the choice of the level of detail which should be both feasible and relevant. The quantification of rates, environmental influences and interrelationships in the system will be a challenge.

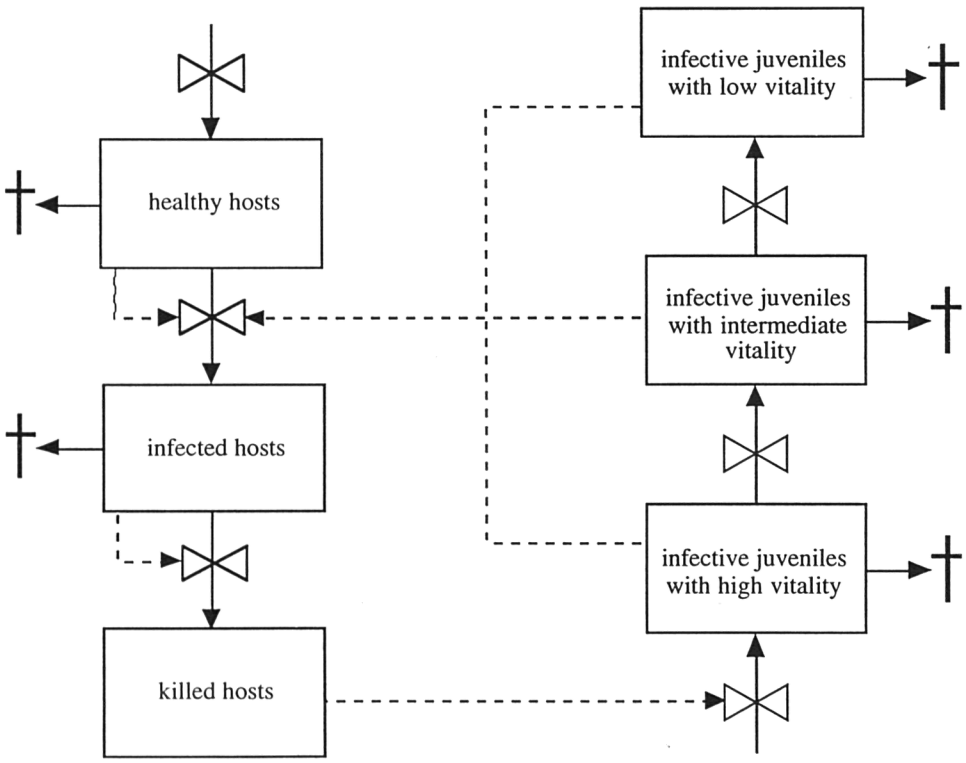

Fig. 2. Relational diagram illustrating the infection process (healthy insect hosts becoming infected and dying subsequently) and the gradual loss of infectivity of EPNs with age. Boxes are state variables. Drawn arrows + valves (-⧖►) represent rates of change of state variables. Hatched arrows indicate that the state variable at the origin of the arrow influences the rate(s) of change of (an)other state variable(s).

PROSPECTS

There is a wealth of information about temperature requirements and optima, survival rates and dose-mortality relationships for a range of hosts for several *Steinernema* and *Heterorhabditis* species, especially *S. carpocapsae* and *H. bacteriophora*. Although not all of the available information is optimally suited for parameterizing a model, there is at least sufficient information available to construct a preliminary model. Despite the knowledge of survival under standardized laboratory conditions, it

is still an unresolved puzzle by which process and in which quantities EPNs are 'lost' in the soil system after spraying. Recoveries directly after spray are seldom higher than 50% and often much lower.

There is little knowledge about the quantitative aspects of movement of EPNs in soil. Different EPNs have different host finding strategies; some are of the lying-in-wait type, others can be characterized as search-and-destroy strategists. The model building will require a quantitative concretization of these behaviours under a range of abiotic conditions, host distribution and quality, and the internal condition of the nematode. Modelling the foraging behaviour will help elucidate what strategies are viable from the nematode perspective and which strategies will enhance biological control under different conditions.

The influence of season on life-history parameters and behaviour is yet unknown as are interactions between EPNs and other soil biota (plant roots, competitors, predators, synergists)

Modelling the ecology and efficacy of EPNs is a challenging task. A full and detailed parameterization of the system will be impossible because it would require decades of work to quantify all the relevant relationships for the whole range of environmental conditions, even for a single nematode-host interaction. Research priorities must be set. This can be done with the aid of a preliminary model. Building such a model with literature data will help in structuring the information available and locating essential knowledge gaps. Sensitivity analysis of such a model will demonstrate which of the processes and parameters have the greatest influence on nematode ecology and efficacy and deserve therefore research priority. Modelling and systems analysis thus help to define spear points and allocate tasks in a network of research groups and industries interested in the development of EPNs as biocontrol agents. Models as the intermediate and end-products of systems analysis will provide unique tools for pinpointing critical factors for biological control success, for designing effective application strategies, and for analyzing the risks of persistence and spread of genetically-modified EPNs in the environment.

REFERENCES

Berryman, A.A., 1991. Population theory: an essential ingredient in pest prediction, management, and policy-making. American Entomologist 37,138-142.

Daamen, R.A., R.W. Stubbs & W. Stol, 1992. Survey of cereal diseases and pests in the Netherlands. 4. Occurrence of powdery mildew and rusts in winter wheat. Netherlands Journal of Plant Pathology 98, 301-312.

de Angelis, D.L. & L.J. Gross (Eds), 1992. Individual-based models and approaches in ecology; populations, communities and ecosystems. Chapman & Hall, New York, 525 pp.

de Moed, G.H., W. van der Werf & P.H. Smits, 1990. Modelling the epizootiology of Spodoptera exigua nuclear polyhedrosis virus in a spatially distributed population of *Spodoptera exigua* in greenhouse chrysanthemums. SROP/WPRS Bull. XIII/5 (1990), 135-141.

de Wit, C.T., 1993. Philosophy and terminology. In: On systems analysis and simulation of ecological processes, with examples in CSMP and FORTRAN. Ed. P.A. Leffelaar. Current Issues in Production Ecology Volume I, Kluwer Academic Publishers, Dordrecht, pp. 3-9.

Fransz, H.G., 1974. The functional response to prey density in an acarine system. Pudoc, Wageningen, 143 pp.

Harrington, R., A.M. Dewar & B. George, 1989. Forecasting the incidence of virus yellows in sugar beet in England. Annals of Applied Biology 114, 459-469.

Hurd, H.S. & J.B. Kaneene, 1993. The application of simulation models and systems analysis in epidemiology: a review. Preventive veterinary medicine 15, 81-99.

Janssen, A. & M.W. Sabelis, 1992. Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of tetranychid mites. Experimental and Applied Acarology 14, 233-250.

Leffelaar, P.A., 1993. Basic elements of dynamic simulation. In: On systems analysis and simulation of ecological processes, with examples in CSMP and FORTRAN. P.A. Leffelaar (Ed.). Current Issues in Production Ecology, Volume I, Kluwer Academic Publishers, Dordrecht, Netherlands, p. 11-27.

Mols, P.J.M., 1993. Walking to survive; searching, feeding and egg production of the carabid beetle *Pterostichus coerulescens* L. (= *Poecilus versicolor* Sturm). PhD. thesis, Wageningen Agricultural University, 201 pp.

Nachman, G., 1991. An acarine predator-prey metapopulation system inhabiting greenhouse cucumbers. Biological Journal of the Linnean Society 42, 285-303.

Peters, R.H., 1991. A critique for ecology. Cambridge University Press, 366 pp.

Rabbinge, R. & C.T. de Wit, 1989. Systems, models and simulation. In: R. Rabbinge, S.A. Ward & H.H. van Laar (Eds): Simulation and systems management in crop protection. Pudoc, Wageningen, p. 3-15.

Sabelis, M.W., 1981. Biological control of two-spotted spider mites using phytoseiid predators. Part I. Agricultural Research Reports 910, 242 pp.

Sigmund, K., 1993. Games of life. Explorations in ecology, evolution and behaviour. Oxford University Press, Oxford, 244 pp.

Snedecor, G.W. & W.G. Cochran, 1989. Statistical methods. 8th Ed. Iowa State University Press, Ames, Iowa, 503 pp.

van den Bos, J. & R. Rabbinge, 1976. Simulation of the fluctuations of the grey larch bud moth. Pudoc, Wageningen, 83 pp.

van der Werf, W., W.A.H. Rossing, R. Rabbinge, M.D. de Jong & P.J.M. Mols, 1989. Approaches to modelling the spatial dynamics of pests and diseases. In: R. Cavalloro and V. Deluchi (Eds): PARASITIS 88, Proceedings of a scientific congress, Barcelona, 25-28 October 1988, p. 89-119.

van der Werf, W., R. Rabbinge & W. Heijbroek, 1986. Spring invasion of *Heterodera schachtii* in sugarbeet; a simulation study. Netherlands Journal of Plant Pathology 92, 33-42.

van der Werf, W., G.H. de Moed & P.H. Smits, 1991. A comprehensive simulation model of the epidemiology of *Spodoptera exigua* nuclear polyhedrosis virus on beet armyworm in glasshouse chrysanthemums. IOBC (SROP/WPRS) Bulletin XIV/7, 108-117.

van Roermund, H.J.W. & J.C. van Lenteren, 1993. The functional response of the whitefly parasitoid *Encarsia formosa*. Bulletin IOBC/WPRS 16, 141-144.

Wallace, H.R., 1963. The biology of plant parasitic nematodes. Edward Arnold, London, 280 pp.

THE ROLE OF ENTOMOPATHOGENIC NEMATODES IN REGULATING THE ABUNDANCE OF PEST SPECIES: A GENERALISED MODEL

Peter Hudson[1] & Rachel Norman[2]

[1] Department of Biological and Molecular Sciences, University of Stirling, Stirling FK9 4LA, Scotland

[2] Department of Applied Mathematics and Theoretical Physics, University of Liverpool, PO. Box 147, Liverpool L69 3BX, England

SUMMARY

Entomopathogenic nematodes provide a potential control agent for invertebrate pests and may be capable of regulating the abundance of these pests in the long term. This paper examines the conditions when entomopathogenic nematodes may be capable of regulating invertebrate pests. Unlike most nematodes, entomopathogenic nematodes exhibit the characteristics of microparasites and we apply a basic microparasite model to the system. Two models are developed, Model 1 considers the situation for heterorhabditid nematodes and Model 2 for steinernematid nematodes. In both systems, regulation occurs in pest species at high density when there is a high contact rate between parasite and host and each host produces abundant infective stages that have a high life expectancy. Overall, heterorhabditid nematodes are more likely to be effective control agents than steinernematid nematodes since only one nematode is necessary for infection.

INTRODUCTION

The role of parasites in regulating the size of their host population is a subject that has, in the past, attracted a certain amount of controversy. On the one hand, ecologists believed that parasites should be benign and have little impact on their host population since a parasite that killed its host would also die and thus be at a selective disadvantage. On the other hand, medical, veterinary and parasitological workers had a different perception and by definition classed parasites as species that were harmful to their hosts. Even so, they were more concerned with the effects of the parasite on an individual host rather than the overall effects on the size of the population. The situation was clarified when Anderson and May (1978, 1991) synthesised the principles of population biology with parasitology and demonstrated that infection and the impact of parasites would increase with host density and the conditions when parasites could be expected to regulate a host population. Fitness of parasites does not depend on virulence alone but more specifically on the number of successful infections produced by a parasite.

The analyses by Anderson and May showed that parasites of moderate virulence rather than those of high virulence will tend to have the largest impact on the equilibrium size of the host population. This may appear counterintuitive at first, until one considers that the death of a host also leads to a fall in the size of the parasite population and reduced transmission. When virulence is high, the death rate of hosts and parasites is high and the parasites are lost from the system before

successfully infecting another host. Parasites of moderate virulence will tend to cause morbidity in their hosts, reducing their fecundity and survival but, since the hosts stay alive for longer, transmission is more likely.

There are exceptions to the general rule of moderate virulence and the regulation of host population size. These will occur when transmission occurs after the death of the host. For example, *Capillaria hepatica* is a virulent nematode parasite of wood mice which is dependent on host death for transmission. The eggs of the parasite are deposited in the liver of the mouse and are only liberated by decomposition, cannibalism, necrophagy or predation (Singleton and McCallum, 1990). Entomopathogenic nematodes are a further example where transmission only occurs after the host has been killed so the impact on the host population will not depend on parasite virulence.

This paper addresses the question "Under what conditions will entomopathogenic nematodes regulate invertebrate host species ?" by producing a simple generalised model. The framework of parasite-host models that have been developed depend on the life cycle of the host and have generally been classified as either microparasite or macroparasite models but the entomopathogenic nematodes do not sit clearly within either definition.

CLASSIFICATION OF ENTOMOPATHOGENIC NEMATODES MACROPARASITES OR MICROPARASITES?

Following the protocol of Anderson and May (1978) many epidemiologists consider that parasites fall into two distinct types, the microparasites and the macroparasites. Differences between these two groups relate more to biological differences than to simple differences in size. Macroparasites (helminths and arthropods) in general do not multiply within their host and transmission is achieved through an infective stage. Microparasites are generally much smaller (viruses, bacteria and protozoa), they possess the ability to multiply within their hosts and do not have special transmission stages. As a consequence of these life history differences, two general types of population models have been produced. The models for microparasites are based on a classification of the host population into distinct classes (e.g. susceptible, infected and infectious). The models for the macroparasites considers both the host and parasite population and in particular the distribution and abundance of parasites within the host population.

As a nematode, we may expect the entomopathogenic nematodes to fall within the classification of a macroparasite but they also share a number of characteristics with the microparasites. They multiply within the host and cause severe mortality like many microparasites but are transmitted between hosts by an infective stage like the macroparasites (Table 1). Since there are two distinct classes of host, susceptible and infected and all infections are lethal, we developed the basic model from the microparasite model.

Entomopathogenic nematodes also show some characteristics similar to parasitoids. Infections of both tend to cause mortality of the host as a necessary part of the life cycle and both are parasites of larval stages of invertebrates. However, in entomopathogenic nematodes it is the juvenile stage that seeks the host and the

adult stages that are parasitic whereas in the parasitoid, it is the adult stages that seek the hosts and the larval stages that are parasitic.

TABLE 1. Comparative aspects of the life history of microparasites, macroparasites and parasitoids in relation to entomopathogenic nematodes.

	Micro-parasites	**Macro-parasites**	**Parasitoids**	**Entomo-pathogenic Nematodes**
Direct reproduction within host	Yes	No	No	Yes
Transmission through larval stage	No	Yes	No	Yes
Ratio of body size (parasite:host)	<<1	<1	< or ~1	<1
Ratio of generation time (parasite:host)	<<1	<1	<1	<1
Main impact on host	Mortality	Morbidity	Mortality	Mortality
Ratio of intrinsic growth rate (host:parasite)	<<1	<1	~1	<<1 & <1

ENTOMOPATHOGENIC NEMATODES AND THE REGULATION OF THE HOST POPULATION

The entomopathogenic nematodes act like virulent microparasites, infecting a host, multiplying within the host and, as a consequence of the infection, causing rapid and certain death even though death is caused by the associated bacterial infection rather than the direct actions of the nematodes. As such we develop a modified microparasite model which includes changes in the size of the susceptible and infected host population but also considers the population of free-living juveniles (Fig. 1).

The invertebrate hosts are produced at a density dependent birth rate *a* and are lost from the population at mortality rate *b* such that the host population will rise to carrying capacity K when the death rate equals the birth rate. The hosts become infected with juveniles at rate *B*. The infected cadavers are lost at rate α, produce juveniles at rate λ which die as free-living juveniles at rate μ. The basic life cycle is shown schematically in Figure 1 with further details of notation presented in Table 2.

The model is a simple, generalised model designed to cover the life cycle of entomopathogenic nematodes but one that can be developed and applied to specific systems. The model carries a certain number of assumptions; for example it takes no account of the spatial distribution of infective juveniles, it assumes that all infected hosts die and produce juveniles, there is no seasonal effect and that infected hosts die before producing offspring.

Fig. 1. Schematic representation of the life cycle of entomopathogenic nematodes illustrating basic death and birth rates used in the model.

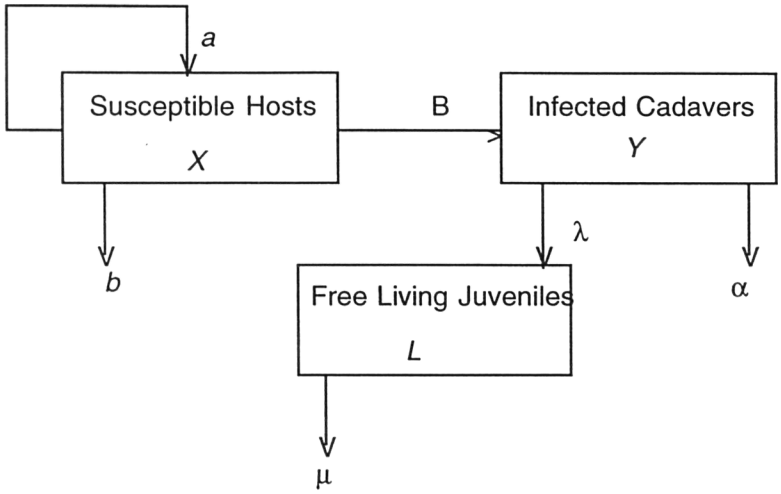

TABLE 2. Notation of parameters used in the basic model.

Symbol	Definition
X	Susceptible host density
Y	Infected cadaver density
L	Free living juvenile density
a	Birth rate of hosts per capita
b	Death rate of hosts per capita
B	Transmission rate of nematodes.
α	1/duration of time before emergence of juveniles
μ	Mortality rate of nematodes = 1/life expectancy
λ	Rate at which nematodes are produced from infected host
K	Carrying capacity of host population

The model itself consist of four, coupled differential equations describing changes in the size of the susceptible host population (X), the infected host population (Y), the total host population ($H = X+Y$) and the size of the free-living juvenile population (L). We consider two scenarios, first Model 1 is the simpler case of the typical heterorhabditid nematodes where we assume that only one nematode is needed for infection. Here:

$$(1) \quad \frac{dX}{dt} = r(1-\frac{H}{K})X - BLX$$

$$(2) \quad \frac{dY}{dt} = BLX - \alpha Y$$

$$(3) \quad \frac{dL}{dt} = \lambda Y - BLX - \mu L$$

$$(4) \quad \frac{dH}{dt} = r(1-\frac{H}{K})X - Y\alpha$$

The second situation describes the case for the steinernematid nematodes where at least 2 infective stages are needed to produce a successful infection. In this instance equation (3) becomes:

$$(5) \qquad \frac{dL}{dt} = \lambda Y - BLH - \mu L$$

In both Model 1 and Model 2, regulation of the host population will occur when:

$$BK\lambda > BK\alpha + \mu\alpha$$

In biological terms this means that if an entomopathogenic nematode is to regulate an invertebrate pest species, it must produce large numbers of nematodes from each infected host (λ large) and the free living juveniles must live for a long period of time (μ small). Transmission rate should be high (B large) which in effect means a high contact rate between infective larvae and hosts, a high probability of the host being infected when they come into contact and few nematodes required for a successful infection. Interestingly regulation will also be high when it takes a long time for the larvae to emerge from the cadaver and the pest species should have a high carrying capacity, K.

DISCUSSION

This short paper describes an initial model for entomopathogenic nematodes and addresses the question "Under what conditions will entomopathogenic nematodes regulate invertebrate pest species?".

To be effective, the host population should be at a relatively high density and there should be a high contact rate between parasites and the pest species. By definition, many pest species of agricultural crops are at high densities. Depending on other variables, few entomopathogenic nematodes may be capable of reducing the size of the pest population to an acceptable equilibrium level. Nevertheless, short term impacts may be severe and be capable of reducing pest density within the time framework of a single crop harvest. High rates of transmission require high rates of contact between host and parasite which may not be possible when hosts are immobile or highly aggregated around crops. Highly mobile pests or highly mobile nematodes would increase the possibility of contact and thus regulation.

Given similar conditions, the heterorhabditid nematodes would be more likely to provide long term control of a pest species since only one nematode is required for infection while two are needed amongst the steinernematids. However these advantages would soon be outweighed if an infection produced more free-living nematodes per infection with a higher survival rate or the juveniles took a long time to emerge from the cadaver, thus helping the persistence of the infection when host densities were low. In real conditions, control may depend more on the suitability and availability of the nematodes and both the short and the long term net benefits.

Future research will investigate in more details the short term impacts of nematodes on their host population, the spatial aspects of host distribution, seasonal effects and the probability of mating success in the steinernematid nematodes.

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REFERENCES

Anderson, R.M. and May, R.M. (1978). Regulation and stability of host-parasite population interactions. I. Regulatory processes. J.Anim.Ecol. **47**, 219-247.

Anderson, R.M. & May, R.M. (1991). Infectious diseases of humans. Dynamics and control. Oxford University Press, Oxford.

Singleton, G.R. and McCallum, H. I. (1990). The potential for *Capillaria hepatica* to control mouse plagues. Parasitology Today **6**, 190-193.

CAUSES AND CONSEQUENCES OF LIFE HISTORY VARIATION IN PARASITIC NEMATODES

Andrew F. Read[1,2] and Arne Skorping[1]

[1]Department Ecology/Zoology, Institute of Biology and Geology, University of Tromsø N-9037, Norway. [2] Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, Scotland.

SUMMARY

Nematodes exhibit enormous life history diversity, yet the evolutionary causes and ecological consequences of this diversity are poorly understood. The diversity is certainly associated with phylogeny, and some broad habitat categorisations such as parasitism. Gastrointestinal nematodes of mammals range from small, short lived, slow growing, early reproducing, low fecundity organisms to those with the opposite suite of characters. Why this pattern of covariation exists is not clear; it is quite different to that of all other groups for which similar analyses have been done. Associating parasite life history with host features has not proved easy, although habitat within a host may affect growth rate and account for phenomena such as larval migration by parasitic nematodes of mammals. Life history is likely to relate to a variety of population dynamical phenomena of interest in biological control, such as persistence and invasion, but there has been little work in the context of nematodes. Parasite reproductive rates are also intimately connected with virulence and recent work indicates the potential of the area of research. Careful experimental work on nematodes as model systems has the potential to illuminate both theoretical issues as well as those associated with biological control and animal and human health.

INTRODUCTION

Two things about nematodes are particularly striking to evolutionary ecologists. They occupy a huge diversity of habitats, despite their relatively invariant body, and they exhibit a truly staggering array of life history strategies. The soil-dwelling *Caenorhabditis elegans*, for example, produces about 300 eggs during the four days of its reproductive life span. In contrast, *Ascaris lumbricoides*, which reproduces in human gastrointestinal tracts, produces probably 65 million eggs during its year long life span (Crompton and Pawloski, 1985) - an average of about two per second. This diversity far outweighs that exhibited by taxa with which life history theorists typically work (eg. birds and insects). But despite these observations, the evolutionary causes and ecological consequences of nematode life histories have been subject to surprisingly little attention by evolutionary ecologists. This oversight is all the more surprising given that fecundity and generation time are likely to be linked - directly or indirectly - with the rate at which organisms extract energy from their habitat and their ability to persist as a population and proliferate into unexploited habitats. In the context of parasitism,

the focus of much nematological research, life history variation has profound consequences for pathology, virulence and epidemiology (Calow and Jennings 1974; Calow, 1983; Anderson and May, 1991). Unless we can understand how natural selection has shaped the life history diversity we see, we can not begin to predict the consequences of artificial selection imposed by medical intervention or the release of nematodes for biological control. Nor can we begin to interpret the natural experiments already underway.

In this paper we attempt to review the causes and consequences of interspecific variation. We are only too aware that we raise more questions than we answer; we believe this is a measure of the infancy of the field (rather than a measure of our ignorance!). Indeed, in many cases the diversity is not yet described well enough to even begin to make sense of it. We focus for the most part on parasitic nematodes of mammals, because we are most familiar with them and because they exhibit some of the greatest diversity. However, work associated with nematodes as biological control agents has the potential to cast light on many of the issues we raise. We hope to provide an introduction into the relevant literature for those interested in this possibility. General reviews of life history theory are provided by Roff (1992) and Stearns (1992); the best dealing with invertebrates is by Godfray (1987) which primarily concerns insects.

THE NATURE OF THE VARIATION

Phylogeny

Although, size, fecundity and age at first reproduction clearly vary within nematode species depending for example on host factors such as immune status, age and sex, this variation is usually relatively small compared with the huge differences typically found across species. Most of the variation is found at higher taxonomic levels. Figure 1 shows the distribution of the variance in some life history traits of gastrointestinal nematodes of mammals. Most of the diversity occurs as differences between higher taxa rather than as differences between species within taxa: over 50% of the variance occurs as differences between families or higher taxa. This is obvious despite the bias that measurement error inflates the within-genera variance. This bias probably accounts for the relatively high within-genus variance in patency (reproductive life span), which is perhaps the most difficult variable to estimate reliably.

In many other groups such as mammals, birds and reptiles (reviewed by Stearns, 1992), life histories typically differ more between taxa (rather than within them). This pattern is interesting in its own right and its evolutionary significance is not clear, implying as it does that most of the variation arises as the taxon is radiating, with only minor variations being added later. Whatever the explanation, it has formidable implications for analysing interspecific life history variation, because it implies that species can not represent independent data points in analysis, since closely related species are more likely to be similar than less related species. Thus coincidental differences between taxa can become inflated into apparently significant differences because of repeated sampling of species within those taxa. This generates a problem of pseudoreplication of taxon-specific effects. This is now well understood in a number of contexts and is the subject of considerable

methodological research (Harvey and Purvis, 1991; Read and Nee, in press). Space prevents further discussion of this issue here; those interested in further details could start with Harvey and Pagel (1991) and in the context of life history variation with Harvey and Keymer (1991), Harvey *et al.* (1989) and Stearns (1992). Suffice to say it is simply no longer good enough to analyse life history variation using species points as independent.

Methods to overcome these statistical issues share the common feature that they use phylogenetic hypotheses to control for non-independence. In the absence of any alternatives, our own research on nematode life histories derives phylogenies from morphological systematics. It remains an open and critical issue whether many (or even some) of the traits on which such work has been based are convergent; there is an urgent need for higher level molecular systematic analysis of nematode taxa.

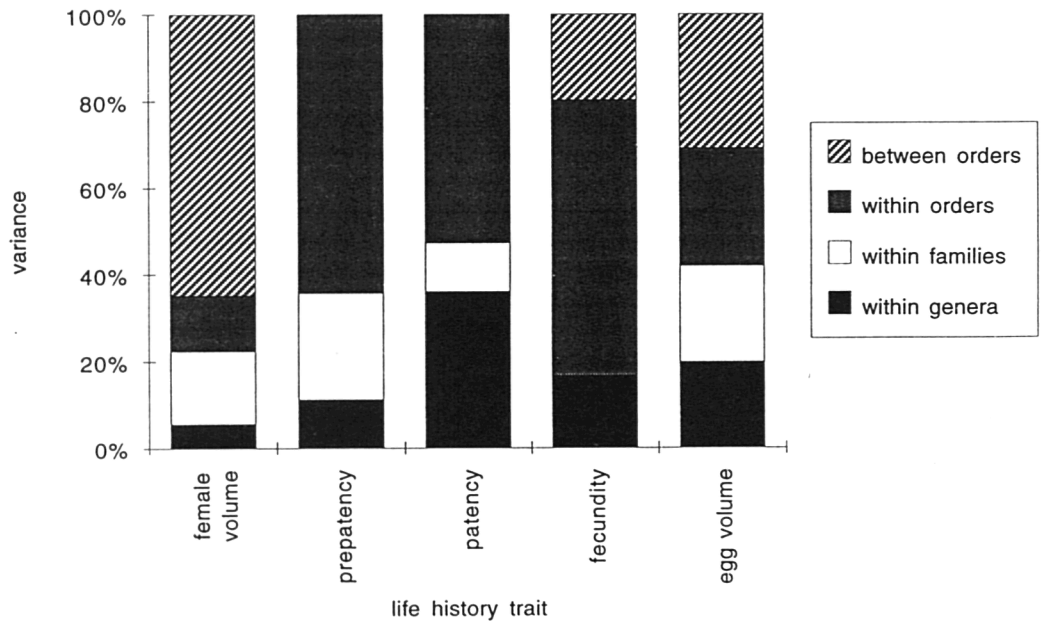

Fig. 1. Proportion of variance in various life history traits of gastrointestinal nematodes found at various taxonomic levels. Thus, of the variation in female body volume found across species, 5% is found within genera, 65% between orders etc. Data and sources from Skorping *et al.* (1991).

Size

As with many invertebrate groups (Blueweiss *et al.*, 1978), body size is a critical life history variable in nematodes. Bigger nematodes take longer to begin producing eggs, but are more fecund (Figs. 2 and 3) and may live for longer. This makes body size an important target of selection. However, this does not necessarily mean that life history diversity is some sort of allometric consequence of size (cf. Huxley, 1932; Blueweiss *et al.*, 1978; Stearns 1992), where reproductive rates are "allometrically constrained within a narrow envelope of possibility by size" (Western and Ssemakula, 1982). As is obvious from the figures, there is no "narrow window": species can have, for example, similar prepatent periods but body volumes which differ by up to four orders of magnitude. The implication is that size, along with fecundity and age to maturity, is subject to selection because of its life history consequences, and that such multidimensional selection pressures produce the patterns of covariation we see (Read and Harvey, 1989; Harvey *et al.*, 1989; Skorping *et al.*, 1991).

Life History Covariation

The few previous attempts to make sense of life history variation within groups of endoparasites (reviewed by Skorping *et al.*, 1991) have been largely based on theory concerning r- and K- selection (MacArthur and Wilson, 1967; Pianka, 1970; see Begon *et al.*, 1990 for critical review) . This theory is now widely appreciated as inadequate on both empirical and theoretical grounds and is at best a special case of more general age-specific demographic models of life history evolution (Charlesworth, 1980; Boyce, 1984; Partridge and Harvey, 1988; Stearns, 1992). The traditional interpretation of r/K theory (Pianka, 1970) has been to predict a continuum from small, fast growing, highly fecund, short lived organisms (r-) to large, slow growing, low fecundity, long lived organisms. The extent to which this pattern was actually predicted as opposed to being fitted to mouse to elephant curves is unclear, but whatever its theoretical explanation, such a continuum has been found in taxa as diverse as mammals, birds and reptiles. This continuum is not, however, typical of gastrointestinal nematodes of mammals, the only group of nematodes so far examined (Skorping *et al.*, 1991). In this group there is a continuum from small nematodes with short developmental periods, low somatic growth rates, low fecundity and short reproductive periods (like the trichostrongyles) to large species (like the ascarid nematodes) with long developmental periods, high average growth rates, high fecundity and long reproductive life spans.

CAUSES OF THE VARIATION

Habitat

Given both the great diversity of habitats occupied by nematodes and the diversity of their life history strategies, it is tempting to expect habitat and life history to be causally linked. Indeed, the idea that habitat is the templet for life history evolution (Southwood, 1988) has been pervasive in ecology, and there has been a proliferation of schemes attempting to classify habitats likely to produce selection

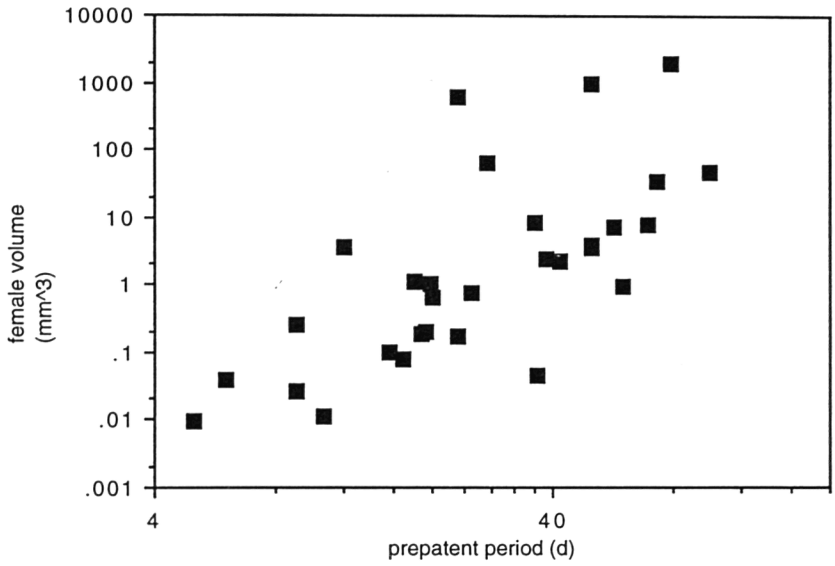

Fig. 2. Log-log plot of the association between prepatency period (time from infection to release of first eggs/larvae) and female body volume for gastrointestinal nematodes of mammals. Plotted points are generic means. Data sources and analysis from Skorping *et al.* (1991).

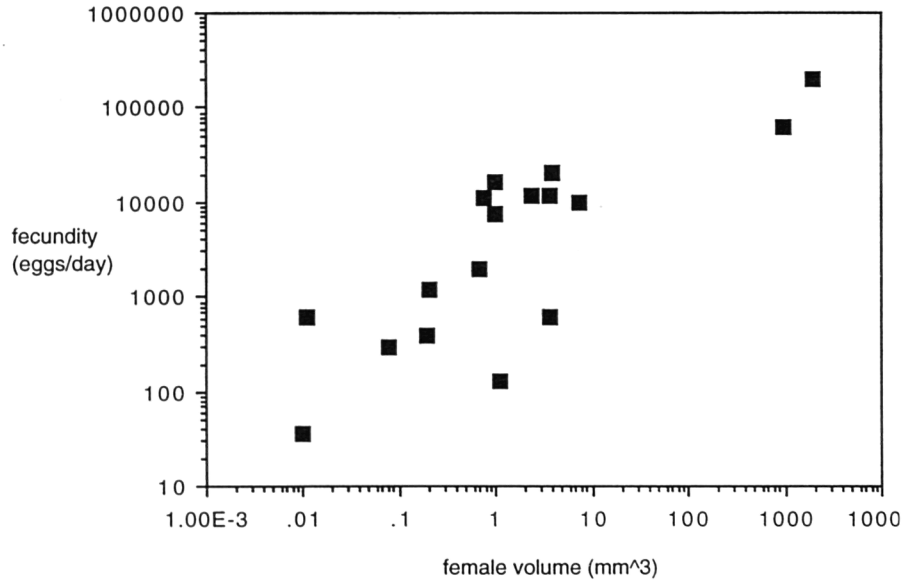

Fig. 3. Log-log plot of the association between female body volume and fecundity for gastrointestinal nematodes of mammals. Plotted points are generic means. Data sources and analysis from Skorping *et al.* (1991).

pressures generating similar life history strategies (reviewed by Begon *et al.* ,1990; Stearns, 1992; Roff, 1992). Yet with few exceptions, attempts to correlate habitat variation with interspecific variation in life histories has proved to be largely unsuccessful in other taxa (reviewed by Partridge and Harvey, 1988; Stearns, 1992) and indeed, so far as we are aware, there are few rigorous analyses which demonstrate a link in nematodes.

However, one pattern does seem sound: nematodes which are parasitic are larger and more fecund than their free-living relatives (Calow and Jennings, 1974). For instance, *Strongyloides ratti* is a nematode with life cycle which can alternate between free-living and parasitic. Inside a rat, daily worm fecundity can exceed 50 eggs/day; in contrast, free-living females produce only 15 eggs/day. The enormous fecundity of parasitic nematodes is frequently thought of as an adaptation to parasitism which is forced on obligate parasites by high rates of egg and larval mortality outside hosts and the low probability of locating hosts (eg. Maizels *et al.*, 1993). This is perhaps one of the most widely held misconceptions about parasite life histories. Even if transmission between hosts is an uncertain process requiring large fecundities (and we note that many parasitic nematodes persist even with low fecundity), that can not explain the difference between free-living and parasitic: selection should favour highly fecund worms whether or not they were parasitic. A more likely explanation for the difference is that suggested by Calow and Jennings (1974): parasitic worms probably live in a more energy rich environment and can thus achieve greater fecundities. This would also provide for a selective benefit perhaps sufficient to account for the evolution of parasitism in the first place.

More recently, we have discovered that mammalian nematodes whose larvae develop in the tissues are large and more fecund than related taxa whose larvae develop only in the gastrointestinal tract (Read and Skorping in prep.). This pattern is independent of the adult habitat, life cycle, host diet, size or generation time. It apparently arises because worms grow faster if they have a tissue phase. As yet we have no explanation as to why this should be so, although we have a number of speculations. For instance, worms in the tissues are bathed in processed nutrients in an aerobic environment; worms in the gastrointestinal tract are subject to harsher biochemical conditions. Whatever the cause of the pattern, it has a number of implications. One is that it provides the first adaptive explanation for larval migration in nematodes. Many oral infecting species arrive in the gastrointestinal tract, penetrate into the tissues and undergo often extensive migrations through the host, only to return to the gastrointestinal tract to breed. These migrations are frequently associated with considerable pathology. Our view is that these migrations are analogous to the pre-spawning migrations of salmon: migration is favoured by selection because it enables growth to occur in more benign conditions, so that greater adult size (and hence fecundity) can be achieved. In any case, our analyses point to the importance of *intra*-host habitat as an influence on life history diversity in nematodes.

Size

Other habitat correlates would be worth investigating. For instance, Anderson (1992) suggests that nematodes using intermediate hosts are larger when they infect their final host. This might provide an explanation for the evolution (paradox) of heteroxeny (if successfully infecting a single host is unlikely, successfully

infecting two or more should be extremely unlikely). Body size of the definitive host is also an obvious candidate correlate. For purely mechanical reasons, small hosts may be unable to harbour large (and therefore fecund) worms. Similarly, at least amongst mammals, larger hosts live longer (Read and Harvey 1989) and worms which delay reproduction in order to reap the fecundity benefits of greater growth will be less likely to suffer from host mortality. Indeed, amongst pinworms (*Enterobius*) of primates, larger worms are found in larger hosts. Further analysis showed that this relationship was primarily due to host longevity rather than host size *per se* (Harvey and Keymer, 1991). Exactly why this should be so is unclear: *Enterobius* typically have considerably shorter lives than their primate hosts. Our investigations of host body size demonstrate that across a broader range of nematodes, host body size has rather little explanatory power (Skorping and Read in prep.) It is indeed the case that small hosts do not harbour the largest nematodes like *Ascaris*, but host size is rather a poor predictor of worm size: horses, for example, are host to almost the full range of worm sizes found in mammalian nematodes. We have not yet analysed the confounding effects of host taxonomy or diet, both of which might be interesting predictors of nematode life history diversity in their own right.

Covariation

Why should mammalian gastrointestinal nematode life histories (see above) be arranged from small, early reproducing, low fecundity, short-lived species to species with the opposite suite of traits? We have suggested (Skorping *et al.*, 1991) that there is a threshold level of investment per egg above which the reproductive success of that egg is not increased. If so, then extra resources should be invested in more eggs. Hence larger worms, which will take longer to grow, will be more fecund. This would contrast with other groups, such as mammals and birds, where extra resources are frequently used to produce higher quality offspring, thus producing a different pattern of life history covariation. Analyses of free-living nematode taxa or other invertebrate phyla are clearly warranted. It would also be of great interest to know what the picture is for nematodes parasitising other habitats.

CONSEQUENCES OF THE VARIATION

Persistence/Colonisation

Implicit in the idea that high fecundity and rapid age to maturity allows rapid population growth (Cole, 1954; Stearns, 1992) is the idea that species with such traits should be able to rapidly colonise and exploit new niches (e.g. susceptible host populations). Likewise, long lived species, or those with long lived, highly resistant stages, may be able to better survive stochastic population fluctuations. These sort of ideas are prevalent in ecological theory (e.g. Pimm, 1991). There has been some attention paid to the effects of life history strategy for conservation biology (principally, extinctions of slowly maturing, low fecundity, endemic island fauna, e.g. King, 1984). However, to our knowledge they have been subject to no investigation in the case of nematodes or parasites in general. Epidemiologists typically consider all well studied human nematodes to be at population equilibrium which is unrelated to life history strategy (Anderson and May, 1985) as a consequence of density dependence. Entomophagous nematodes offer

unprecedented possibilities for experimental investigation of the importance of life histories on population dynamics. One good example of what may be possible comes from *C. elegans*, where a mutant producing more eggs than wild type worms has been found (Hodgkin and Barnes, 1991). Why hasn't this mutation swept to fixation? Evidently this is because it does not have an advantage in periods of rapid population growth, because it achieves greater fecundity at the cost of longer generation times (it takes longer to produce the extra sperm involved). This system would provide an ideal model to investigate the trade-off between traits useful during periods of rapid population expansion (short generation time) and those useful at equilibrium (higher lifetime reproductive success).

Virulence

Virulence can have substantial effects on the genetic contribution of both host and parasite to the next generation. Why there is variation in the virulence of infectious diseases has been the subject of much recent theoretical work by evolutionary ecologists (for a recent overview, see Read, 1994), but unfortunately, this theoretical work has far outstripped the empirical base. Probably for historic reasons, virulence is considered primarily a parasite trait in this literature, though much pathology associated with eukaryotic parasites is clearly a consequence of host responses. Furthermore, in the case of nematodes which shed infective propagules outside the host but do not replicate within it, pathology is a function of parasite burden; this is likely to be determined by factors extrinsic to any single worm. Virulence thus can be considered as primarily a parasite trait only to the extent that worms alter their detrimental effects on the host in response to total worm burden. We know of no evidence to support that.

Those caveats aside, it nevertheless seems likely that nematode virulence will be associated at least in part with life history variation. Worms that are larger and more fecund must be extracting more resources from hosts, and we mentioned above that larval migration in mammalian tissues, which we have argued is an adaptive life history strategy by the nematodes, results in considerable host damage. Given that biological control is necessarily about virulence, work with entomopathogenic nematodes offers unprecedented experimental possibilities for the analysis of nematode virulence. However, so far as we are aware, there has only been one empirical study of the selective basis of nematode virulence. Herre (1993) showed that species of *Parasitodiplogaster* each of which parasitises a specific fig wasp species, were less virulent when the natural history of their host meant that vertical transmission was a larger component of worm fitness. In this system, life history decisions by the worms are intimately connected to virulence: it is assumed that extracting host resources so as to increase worm fecundity decreases host fecundity. The finding accords well with theory: when parasite fitness is intimately linked with host fitness, selection should favour more benign parasites. As host and parasite fitness become progressively uncoupled, the negative effects of parasites become stronger. However, we have little detailed understanding of why virulence and vertical transmission are coupled in the way Herre reported. For instance, the pattern accords well with theory qualitatively, but we have no understanding of why the quantitative relationship should be as it is. In addition, was the effect due to selection imposed by intra-host competition, or would it have occurred even if hosts were infected with genetically identical

worms? Teasing apart those and related issues should be possible with the appropriate system, and is essential if we are to really test our understanding of the evolution of virulence (Read *et al.* in press).

CONCLUSION

We hope that two messages emerge from this review. First, that not a lot is known and that much of what is asserted is speculative or based on largely anecdotal reports. Second, as the work of Hodgkin and Barnes (1991) and Herre (1994) demonstrates, much of general interest can be learnt by appropriate analyses of amenable systems. Entomopathogenic nematodes offer considerable possibilities in this regard. Given the applied interest in traits closely linked with effective biological control (epidemiology, virulence), we believe there are considerable possibilities for those wishing to grasp the nettle. Currently, evolutionary ecologists can make few practical suggestions about problems of the population dynamical issues of invasion, spread and persistence, or of how virulence might be expected to evolve. We believe this is because there is little interaction between theory and applied problems; that this should be so when there are obvious benefits to collaboration is all the more strange.

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REFERENCES

Anderson, R. C. (1992). *Nematode Parasites of Vertebrates. Their Development and Transmission.* CAB International, Wallingford, UK

Anderson, R. M. and May, R. M. (1985). Helminth infections of humans: mathematical models, population dynamics and control. Adv. Parasitol. **24**, 1-101.

Anderson, R. M. and May, R. M. (1991). *Infectious Diseases of Humans. Dynamics and Control.* Oxford University Press, Oxford

Begon, M., Harper, J. L. and Townsend, C. R. (1990). *Ecology. Individuals, Populations and Communities.* Blackwell, Oxford

Blueweiss, L., Fox, H., Kudzma, V., Nakashima, D., Peters, R. H. and Sams, S. (1978). Relationships between body size and some life history parameters. Oecologia **37**, 257-272.

Boyce, M. S. (1984). Restitution of r- and K- selection as a model of density dependent natural selection. Ann. Rev. Ecol. Syst. **15**, 427-448.

Calow, P. (1983). Pattern and paradox in parasite reproduction. Parasitology **86**, 197-207.

Calow, P. and Jennings, J. (1974). Calorific values in the phylum Platyhelminthes: the relationship between potential energy, mode of life and the evolution of endoparasitism. Biol. Bull. **147**, 81-94.

Charlesworth, B. (1980). *Evolution in Age-Structured Populations.* Cambridge University Press, Cambridge.

Cole, L. C. (1954). The population consequences of life history phenomena. Q. Rev. Biol. **29**, 103-137.

Crompton, D. W. T. and Pawlowski, Z. S. (1985) Life history and development of *Ascaris lumbricoides* and the persistence of human ascariasis. In: Crompton, D. W. T., Nesheim, M. C. and Pawlowski, Z. S. (Eds.) *Ascariasis and its public health significance*, pp. 9-23. Taylor and Francis, London.

Godfray, H. J. C. (1987). The evolution of clutch size in invertebrates. Oxf. Surv. Evol. Biol. **4**, 117-154.

Harvey, P. H. and Keymer, A. E. (1991). Comparing life histories using phylogenies. Phil Trans. R. Soc. Lond. B **332**, 31-39.

Harvey, P. H. and Pagel, M. D. (1991). *The Comparative Method in Evolutionary Biology.* Oxford University Press, Oxford.

Harvey, P. H. and Purvis, A. (1991). Comparative methods for explaining adaptations. Nature **351**, 619-624.

Harvey, P. H., Read, A. F. and Promislow, D. E. L. (1989). Life history variation in placental mammals: unifying the data with the theory. Oxf. Surv. Evol. Biol. **6**, 13-31.

Herre, E. A. (1993). Population structure and the evolution of virulence in nematode parasites of fig wasps. Science **259**, 1442-1445.

Hodgkin, J. and Barnes, T. M. (1991). More is not better: brood size and population growth in a self-fertilising nematode. Proc. Roy. Soc. B. **246**, 19-24.

Huxley, J. S. (1932). *Problems of Relative Growth.* Methuen, London.

King, C. (1984). *Immigrant Killers. Introduced Predators and the Conservation of Birds in New Zealand.* Oxford University Press, Auckland.

MacArthur, R. H. and Wilson, E. O. (1967). *The Theory of Island Biogeography.* Princeton University Press, Princeton.

Maizels, R. M., Bundy, D. A. P., Selkirk, M. E., Smith, D. F. and Anderson, R. M. (1993). Immunological modulation and evasion by helminth parasites in human populations. Nature **365**, 797-805.

Partridge, L. and Harvey, P. H. (1988). The ecological context of life history evolution. Science **241**, 1449-1455.

Pianka, E. R. (1970). On r and K selection. Amer. Natur. **104**, 592-596.

Pimm, S. L. (1991). *The Balance of Nature? Ecological Issues in the Conservation of Species and Communities.* University of Chicago, Chicago.

Read, A. F. (1994). The evolution of virulence. Tr. Microbiol. **2**, 73-76.

Read, A. F., Albon, S., Antonovics, J., Apanius, V., Dwyer, G., Holt, R. D., Judson, O., Lively, C., McLean, A. R., Metz, H., Schmidt-Hempel, P., Thrall, P. and Via, S. (in press) Genetics and evolution of infectious diseases in natural populations. In: Grenfell, B. and Dobson, A. P. (Eds) *Ecology of Infectious Diseases in Natural Populations.* Cambridge University Press, Cambridge.

Read, A. F. and Harvey, P. H. (1989). Life history differences among the eutherian radiations. J. Zool. **219**, 329-353.

Read, A. F. and Nee, S. (in press). Inference from binary comparative data. J. Theor. Biol.

Roff, D. A. (1992). *The Evolution of Life Histories. Theory and Analysis.* Chapman and Hall, New York.

Skorping, A., Read, A. F. and Keymer, A. E. (1991). Life history covariation in intestinal nematodes of mammals. Oikos **60**, 365-372.

Southwood, T. R. E. (1988). Tactics, strategies and templets. Oikos **52**, 3-18.

Stearns, S. C. (1992). *Evolution of Life Histories.* Oxford University Press, Oxford.

Western, D. and Ssemakula, J. (1982). Life history patterns in birds and mammals and their evolutionary interpretation. Oecologia **54**, 281-290.

CONCEPTUAL APPROACHES TO EFFECTS OF INTERACTING HOST SIZE AND DENSITY ON GENETIC DIVERSITY IN ENTOMOPATHOGENIC NEMATODE POPULATIONS.

Martin J. Downes

Department of Biology, St Patrick's College, Maynooth, Co. Kildare, Ireland.

INTRODUCTION

The real influence of entomopathogenic nematodes (EPN) and other entomopathogens, on the stability of particular soils and the biological diversity of vegetation, is just beginning to be investigated. Sand dune soils in particular offer promising settings for such work, because EPN of the two commercially most important genera *Steinernema* and *Heterorhabditis* are of general occurrence there, as are entomopathogenic fungi of two important soil genera *Metarhizium* and *Beauveria.* Furthermore, information about natural dynamics of sand dune systems is of great importance in the devising of conservation measures.

It is generally felt that, both for the purpose of developing better commercial EPN strains and the management of existing strains, there is now a great need for fundamental research aimed at understanding their life strategies and the environmental background against which those strategies operate.

EPN infective juveniles (IJs) are specialised dauer juveniles that emerge in large numbers from insect cadavers and invade new insect hosts. In the case of *Heterorhabditis*, an IJ invading a host will become a self-fertile adult and, feeding inside the dead insect, will produce offspring. These offspring comprise females that are not self-fertile and some males: they reproduce by male X female crossings for one or more generations (depending on the size of the insect) inside the same insect cadaver. The development of the final generation of juveniles in the depleted cadaver is arrested giving rise to IJs. Meanwhile, the individual that originally invaded the host has produced IJs directly, inside its own body; the majority, or all, of these IJs are understood to be produced by self-fertilisation.

Therefore, some of the IJs emerging from any cadaver will have been produced by selfing only, and some of the progeny of each of these will also be produced by selfing only. This will be repeated unendingly over time. Other IJs emerging from the same cadaver will have arisen by male X female crossing, but some of the progeny of each of these will be produced by selfing and will in turn produce some by selfing. This also will be repeated over time, with new perpetually selfed lines arising in each generation (Fig. 1), expected to become highly homozygous with an inbreeding coefficient of 0.999 after 10 generations (Falconer, 1989). I term such homozygous, perpetually selfed lines "pseudoclones" (PCs).

Arising from this form of reproduction, assuming that there is no differential mortality, we would expect large natural populations of *Heterorhabditis* to contain IJs that have

been produced entirely by selfing for every integer number of generations from one to effective infinity. Such populations should also contain IJs that have been produced by selfing followed by none to a few crosses in each host cadaver over countless

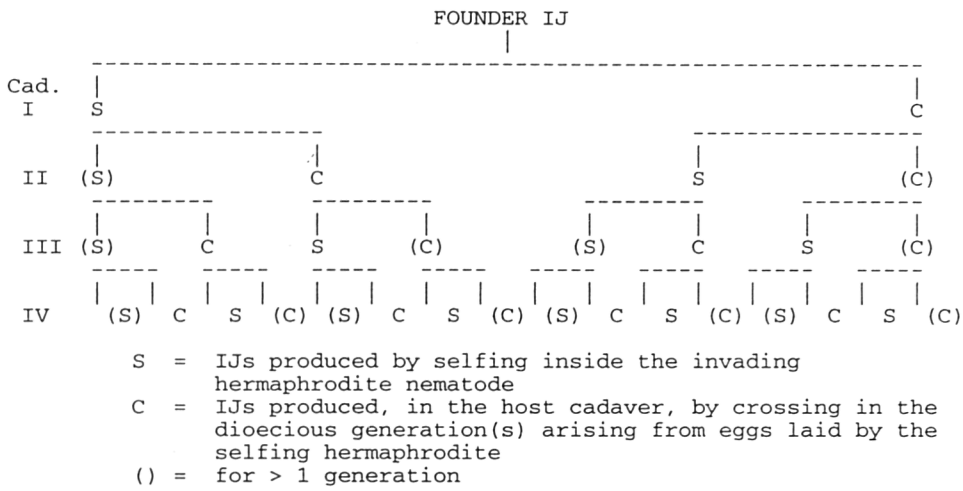

Fig. 1. The lineages of *Heterorhabditis* arising from a single founder IJ cultured through 4 succeeding cadavers.

generations. Between these two extremes, if host size (and therefore the ratio of PCs to crossed progeny produced in any cadaver) varies from time to time, we should expect to find IJs with all relative frequencies of selfing and crossing in their ancestry.

PREVALENCE OF HETEROZYGOSITY IN HETERORHABDITID POPULATIONS

While Maynooth work suggests that *Heterorhabditis* (Irish Group) is often rare where it occurs in Irish sand dunes, there clearly is sufficient diversity in many, if not all, *Heterorhabditis* populations: lines showing very diverse characteristics have been produced in in-breeding programmes (Glazer *et al.*, 1991) even using laboratory-maintained cultures. Such cultures, maintained in relatively constant laboratory conditions for some time, and possibly put through frequent genetic bottlenecks, might be expected to be more genetically depauperate than wild populations. It seems then, that despite the frequent apparent rarity of IJs in soil, to which self-fertility is sometimes seen as a necessary adaptation because of the difficulty of finding a mate, sufficient genetic exchange takes place in natural populations to maintain considerable diversity. A probable consequence of this is that the transmission of exotic genes of genetically manipulated heterorhabditids would not be helpfully restricted by rarity of crossing between strains, even where *Heterorhabditis* populations are at low natural levels.

PATCH AND RARITY EFFECTS ON HETEROZYGOSITY

Little genetic difference would be expected to appear between groups of currently randomly mating individuals within a population, that would be directly attributable simply to their having been crossed for different numbers of generations. This is because very few crosses are necessary to restore gene frequency equilibrium (Hardy-Weinberg equilibrium). However, gene frequency differences between groups would be likely to arise as a result of genetic bottleneck effects where populations fall to very low levels at times and mobility is low, restricting the interbreeding in recovering populations to patches. This may very well be the case in those Irish sand dune populations of *Heterorhabditis* we record to be at low frequencies much of the time.

SELFING LINES AND INBREEDING DEPRESSION

Since new PCs are expected to be generated each time an IJ produced by crossing enters a host, it follows that inbreeding depression should be a constant feature of *Heterorhabditis* populations. Perhaps indeed this factor leads to extinction of PCs on at least some occasions. Experimentally inbred lines at Maynooth differ markedly in their ability to reproduce (Burnell, A. M., personal communication): of course poor reproduction, where it occurs in some such inbred lines, might be due to unfavourable gene combinations and not to the expected doubling of deleterious recessive alleles. Under simple natural conditions, the more competent lines (PCs) would be expected to replace any faulty lines.

Effects of selfing are less well studied in animals than in plants, and reports are sometimes contradictory. The cestode *Hymenolepis nana* is rather like *Heterorhabditis* in that it is believed to use self-fertilisation frequently in nature. Rogers and Ulmer (1962) found that when it was continually selfed its eggs and cysticercoids lost viability and selfed strains could not be maintained beyond 5 generations. By contrast, Nakamura and Okamoto (1993) selfed *H. nana* for more than 20 generations without apparent ill-effect. These workers suggest that, among factors that may be responsible for the conflict of evidence are differences in hosts or strains of *H. nana* used in the different laboratories. It seems obvious that at least some strains of these worms can undergo repeated selfing without obvious inbreeding depression.

We may guess that, like *H. nana*, at least some natural *Heterorhabditis* strains can withstand repeated inbreeding. However, it would be unwise to generalise with too much conviction about the position with natural *Heterorhabditis* strains in the absence of considerably better information about their life strategies in different habitats and the implications of those strategies for the genetic structure of populations. Obviously, knowledge of that structure, especially as it affects genetic diversity and susceptibility to inbreeding depression, would be of extraordinary practical value in the search for lines pre-adapted for commercial multiplication.

Double deleterious recessives should be lost from the population during periods of selfing. Selfing lines (PCs) of *Heterorhabditis* repeatedly arise from parents in the crossing portion of the population and repeatedly contribute new parents to the crossing population: we can therefore see that the genes of some portion of the

crossing population get diverted into selfing lines at each generation and get returned again to the crossing population after some number of generations. Clearly, deleterious recessive alleles might be "filtered out" of the overall population by this process rather as detritus is removed from aquarium water by passing some of the water through a tank filter and then returning it to the aquarium. This "tank- filter" process would be aided by the gentle selection that must operate against deleterious recessives during the selfing to produce dioecious adults, that occurs in every *Heterorhabditis* nematode that infects an insect.

Overall therefore, deleterious recessive alleles are expected to be at low frequencies in heterorhabditid populations.

IN-MIGRATION, HOMOZYGOSITY AND GENOTYPE DIVERSITY

In *Heterorhabditis*, the constantly-arising pseudoclones would be expected to give rise to high diversity of relatively homozygous genotypes in the overall population. A portion of the population would have the ecological characteristics of mixed clone populations. At the same time, much of the male X female crossing that occurs inside a cadaver must occur between siblings. This, like selfing, would be expected to give rise to highly homozygous individuals. The degree of homozygosity in the population overall will depend greatly on the relative frequency with which two or more IJs of different genotypes enter a single insect, permitting crossing between lines. In this connection, the simulation modelling of *Ranunculus repens* (a plant with partly clonal reproduction) carried out by Watkinson and Powell (1993) is probably very relevant. If *R. repens* clones can be seen as usefully similar to perpetually selfing lines of *Heterorhabditis*, the study would suggest that input of only a few individuals carrying new gene combinations into a *Heterorhabditis* population at each generation would accelerate the loss of previously existing PCs but would act powerfully at the same time to maintain genotype (PC) diversity in the population. (In the absence of such input, PC lines would still be lost, but more slowly, and the population would become dominated by just a few PCs).

HOST SIZE AND HOMOZYGOSITY IN EPN

Less obvious perhaps than the effects of crossing between lines, is the influence that the size of the insect host will have on the degree of homozygosity in a *Heterorhabditis* population. The smaller the insect is, the less likely it is to support the development of IJs other than those produced by self-fertilisation within the invading nematode. Therefore, a prevalence of small hosts should result in a greater proportion of PCs in the overall nematode population.

LeBeck *et al.* (1993) have reported that first instar cadavers of the celery leafminer *Liriomyza trifolii* ruptured when invading nematodes grew to be adults. Perhaps very small hosts, in which EPN cannot reproduce at all, are death-traps that play a part in reducing EPN numbers in soil; otherwise we should expect EPN to have evolved the ability to detect and avoid small hosts under natural conditions, or perhaps to fail to penetrate small insects.

ECOLOGICAL STRATEGIES

Populations of *Heterorhabditis* then, can be expected to comprise lines that are effectively clonal and other lines that are mainly cross-breeding. The ecological implications of this difference in the way genes are inherited is not obvious, but it strikes to the heart of one of the greatest issues in fundamental biology: the meaning and value of sex. Perhaps the most immediately relevant aspect of this question for present purposes is the distinction that has been made between the underlying strategies of clonal and sexual populations with respect to competition between those two kinds of population and also with respect to the ability of short generation-time parasites to adapt genetically. While there are several competing hypotheses, the two that are perhaps most popular, in one form or another, would suggest (1) that sex allows EPN to cope with their own small, rapidly adapting parasites or pathogens by producing genetically diverse offspring that make such adapting difficult (the Red Queen hypothesis) and (2) that a sexual population comprising individuals of varying ecological requirements can always find some portions of its ecological range from which it can displace clonal populations which are of narrower ecological competence (the Tangled Bank hypothesis). (These two hypotheses, among others, are discussed in the splendid book by Bell (1982)). For applied purposes however, the question is whether PCs and crossing lines exhibit different strategies that are important to their effectiveness as pest control agents.

PATCH DYNAMICS

The EPN/insect host relationship constitutes a pathosystem that has survived for a very long time: it follows that there are limits to the population fluctuations of each partner. It may be that the host (and consequently the parasite) often becomes extinguished by EPN in patches of its range, as previously suggested by Hominick and Reid (1990), and that these patches are colonised again following decline of EPN numbers. There is rather little information available on this issue, but the size and frequencies of such extinction patches, if they occur, would be a measure of the rate at which the host population declines and of how quickly both host and EPN can migrate into new patches. This will be important information for the evaluation of risk in the release of genetically modified organisms.

INTERACTIONS OF HOST SIZE AND THE DENSITIES OF BOTH HOST AND *EPN*

If extinction patches are very small or non-existent, the interactions of host density and size, that would be expected in principle, will become more obviously central in determining whether the overall pathosystem will persist. In simple situations, assuming that host size is not strongly related to susceptibility to EPN, large hosts, by producing many IJs per infected insect will be expected to lower their own density: host density will therefore be determined by host size. However, if host density were to fall too low the EPN would fail to find the insects and would therefore become extinct: the pathosystem would not persist (except of course by patch dynamics, which we are assuming to be absent for our purposes).

Small hosts on the other hand, by producing few IJs per infected insect, will permit the host to survive at higher densities. At the same time, because scarce insects are difficult for the few IJs to locate, small hosts are actually required to be at higher densities if persistence of the pathosystem is to be insured.

The foregoing is in the nature of a simple analytical model and it makes some predictions: for instance, if EPN are more host-specialised in the wild than we know, kinds with low characteristic densities should parasitise larger hosts and kinds with lower characteristic densities should parasitise smaller hosts. Is it the case that Irish *Heterorhabditis* which so often seems to occur at low frequency in the sandy coastal soils to which it is restricted in Ireland (Griffin *et al.*, 1994), naturally parasitises the relatively large Coleoptera which are common in these dunes? By contrast, perhaps *Steinernema* tends to parasitise small insects in nature. Harris *et al.* (1990) got control of a small dipteran leafminer on crysanthemum in laboratory tests using *S. carpocapsae*. *Steinernema* is always dioecious and this would suggest great dependence on producing varied offspring: this variation will be greater where the "parental" IJs arise from different insect cadavers and are therefore more likely to be distantly related. However, since IJs do not seem to travel far in soil, parasitised cadavers would need to be close together in order that IJs from two or more of them should find a new host and reproduce. Therefore, persistent pathosystems involving *Steinernema* should be expected to possess mechanisms that ensure that hosts remain at high frequencies. One of these mechanisms might be specialization of the EPN on small hosts, another might be a phasing of pathogenicity in the EPN population (see, for example, Bohan and Hominick, this volume). The lesser tendency of *Steinernema feltiae* than of *Heterorhabditis heliothidis* to eliminate insects in mushroom culture reported by Richardson (1987) is in agreement with this hypothesis.

CONCLUSION

We have seen something of how nematode breeding system, host size and host density can be predicted to interact in simple conditions, and how they may affect genetic diversity and heterozygosity in populations of EPN. I hope that this material is provocative, if only in its inadequacy. There is a very serious need for model building on a grander scale than this: currently we have few basic data that would form suitable input for more helpful models.

I have referred to characteristic density, but this has not been well measured: what concerns us mostly in the foregoing discussion is the lowest densities to which host and EPN populations fall, and this has not been measured at all. Most of our information about EPN densities in soil comes from baiting disturbed soil samples with insects such as *Galleria mellonella*; this is a most useful technique but it can tell us nothing about IJs that are in some sense dormant or that have entered soil insects and can perhaps remain in these refugia without reproducing or killing the host for some time, as is reported to be possible in *Lucilia cuprina* under some conditions (Molyneux, 1984). The method described by Spiridonov and Voronov (this volume) could be used to extract dormant or non-infective IJs from soil.

The expected effects of host size on both host and EPN populations would be sensitive to non-random search patterns of EPN in natural soil, as they would be to the degree to which EPN specialize on hosts of different sizes. But we do not really know how EPN find hosts in soil: one of the most impressive things we do know about this is that they are exquisitely sensitive to carbon dioxide gradients, but we find it hard to see how this may form part of effective host location in natural soils containing plant roots and decaying organic matter. We know very little indeed of the bases on which EPN may partition resources under natural conditions, resulting directly or indirectly in host specialization.

It is important that properly integrated models of how EPN behave in natural soil should be produced now (see van der Werf *et al.,* this volume). Since so little is known, and that for widely different systems, it is important that the efforts of groups of workers concerned with this area should be integrated. We need to define the important components of EPN life strategies that can be manipulated, genetically or otherwise, for the greater success of commercial product. Making workable models will encourage integration of effort and enable the most influential components to be recognised.

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REFERENCES

Bell, G. (1982). *The Masterpiece of Nature: the evolution and genetics of sexuality.* Croom Helm, London & Canberra.

Falconer, D. S. (1989). *Introduction to Quantitative Genetics.* 3rd ed., p. 93. Longman, Harlow, Essex.

Glazer, I., Gaugler, R. and Segal, D. (1991). Genetics of the nematode *Heterorhabditis bacteriophora* Strain HP88: the diversity of beneficial traits. J. Nematol. **23**, 342- 333.

Griffin, C.T., Joyce, S.A., Dix, I., Burnell, A.M. and Downes, M.J. (1994). Characterisation of the entomopathogenic nematode *Heterorhabditis* (Nematoda : Heterorhabditidae) from Ireland and Britain by molecular and cross-breeding techniques, and the occurrence of the genus in these Islands. Fundam. appl. Nematol. **17**, 245-253.

Harris, M. A., Begley, J. W. and Warkentin, D. L. (1990). *Liriomyza trifolii* (Diptera: Agromyzidae) suppression with foliar applications of *Steinernema carpocapsae* (Rhabditida:Steinernematidae) and abamectin. J. econ. Entomol. **83**, 2380-2384.

Hominick, W. M. and Reid, A. P. (1990) Perspectives in entomopathogenic nematology. In: Gaugler, R. and Kaya, H. K. (Eds.) *Entomopathogenic Nematodes in Biological Control*, CRC Press, Boca Raton.

LeBeck, L., Gaugler, R., Kaya, H. K., Hara, A. H. and Johnson, M. W. (1993). Host stage suitability of the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). J. Invert. Pathol. **62**, 58-63.

Molyneux, A. S. (1984). The influence of temperature on the infectivity of heterorhabditid and steinernematid nematodes for larvae of the sheep blowfly, *Lucilia cuprina*. *Pest Control: Recent Advances and Future Prospects.* Proc. 4th Austral. Appl. Entomol. Conf., Adelaide. pp. 344-351.

Nakamura, F., and Okamoto, K. (1993). Reconsideration of the effects of selfing on the viability of *Hymenolepis nana*. Int. J. Parasitol. **23**, 931-936.

Richardson, P. N. (1987). Susceptibility of mushroom pests to the insect-parasitic nematodes *Steinernema feltiae* and *Heterorhabditis heliothidis*. Ann. appl. Biol. **111**, 433-438.

Rogers, W. A. and Ulmer, M. J. (1962). Effects of continued selfing on *Hymenolepis nana* (Cestoda). J. Iowa Acad. Sci. **69**, 557-571.

Watkinson, A. R. and Powell, J. C. (1993). Seedling recruitment and the maintenance of clonal diversity in plant populations - a computer simulation of *Ranunculus repens*. J. Ecol. **81**, 707-717.

A MODEL OF HOST-INFECTION BEHAVIOUR FOR THE ENTOMOPATHOGENIC NEMATODE, *HETERORHABDITIS MEGIDIS:* EVIDENCE FOR PRIMARY AND SECONDARY INFECTION STRATEGIES

D.B. Hay[1] and P.H. Smits[2]

[1]Entomology Department, Horticulture Research International, Worthing Road, Littlehampton, West Sussex, BN17 6LP, UK. [2]IPO-DLO, PO Box 9060, 6700-GW Wageningen, The Netherlands.

SUMMARY

Dose/response experiments were done to assess infection of *Galleria mellonella* by *Heterorhabditis megidis* and two models (based on the binomial distribution) were used to analyze the results. The infectivity of this species was relatively low (not more than 40% of the nematodes infected the host in 24 hrs) and varied considerably in replicate experiments. However, initial infection was found to increase the likelihood of subsequent invasion by other nematodes in every experiment. The data suggest that a significant portion of the nematode population fail to initiate infection in unparasitised insects but colonise hosts that are already infected by conspecifics.

INTRODUCTION

In an earlier paper, Hay *et al.* (1994) described the use of an extended binomial model for the interpretation of dose/establishment data for entomopathogenic nematodes. These authors reported that for *Steinernema feltiae*, the probability of primary infection (the likelihood of a first nematode invasion in a hitherto uninfected insect) was less than that for secondary invasion (the probability that an infected host should subsequently acquire additional numbers of parasites). Infected hosts may be more attractive to entomopathogenic nematodes because they leak products of bacterial decomposition (Pye and Burman, 1981) and/or nematode aggregation pheromones (Grewal *et al.*, 1993). Furthermore, preferential invasion of infected hosts may increase the likelihood of mate encounter and may also reduce the risks of host induced mortality.

In this paper, infection behaviour is documented for the nematode, *Heterorhabditis megidis*. This nematode forms hermaphrodite adults in the first generation; thus mate location is an unlikely determinant of infection behaviour for this species.

METHODS

Larvae of the greater wax moth, *Galleria mellonella* were challenged individually with dauer juveniles of *Heterorhabditis megidis* (HE87.3, from The Netherlands) in 50 ml plastic containers filled with sand (8% water content w/w). The nematodes were

produced for experiments in *G. mellonella* and stored at 5°C in tap water for two - six weeks before use. They were applied in 1 ml of tap water and the doses were 1, 2, 4, 8 and 16 dauer stage juveniles per insect larva. There were 30 insects at each dose. The exposure time was 24 hrs at 20°C after which the insect larvae were removed, washed and kept on damp filter paper at 25°C for 5 days before dissection. The number of nematodes in each infected host was then recorded. The experiment was replicated three times (three assays).

There were 30 nematode-free controls in each assay and there was no mortality among these. The dose/establishment data were analyzed using two simple infection models described below.

The simple binomial model.
If any individual nematode has a probability p of establishing an infection then the exact probability that r individuals out of n will invade is given by the binomial distribution:

$$prob(r) = \binom{n}{r} p^r (1-p)^{n-r} = Bin(r, n, p)$$

The extended binomial model.
In the extended binomial model, the simple binomial expression was modified to give two probabilities; the probability of primary invasion (p_1) in uninfected insects and the probability of secondary invasion (p_2), conditional on primary invasion having occurred already. The model is given by:

$$prob(r) = \frac{[1-(1-p_1)^n]}{[1-(1-p_2)^n]} = Bin(r, n, p_2)$$

RESULTS AND DISCUSSION

No more than 40% of the nematodes established in the host in any assay and the overall infectivity was variable (Table 1). For instance, the primary infection probability in assay two was less than half that of assay three suggesting that the infectivity of laboratory populations of *Heterorhabditis*, like Nashes strain of *Steinernema* sp., can exhibit considerable temporal variation during storage at low temperature (Fan, cited in Hominick and Reid, 1990). Furthermore, the relatively low proportion of nematodes that established in the host suggests that many dauer juveniles in the population were non-infectious.

TABLE 1. Infection probabilities for *Heterorhabditis megidis* using two models of invasion.

Assay	Simple binomial model	Extended binomial model	
		Primary (p_1)	Secondary (p_2)
1	0.298 (0.0150)	0.169 (0.0273)	0.360 (0.0261)
2	0.189 (0.1282)	0.092 (0.0179)	0.273 (0.0265)
3	0.271 (0.0146)	0.200 (0.0305)	0.306 (0.0255)

Values in brackets are standard error estimates.

The dose/establishment curves were not consistent in all three assays (Fig. 1). For example, proportional nematode establishment clearly increased at higher nematode doses in assays one and three, but this trend was not obvious in assay two. Nevertheless, the probability of any given nematode successfully invading was significantly higher at doses of two nematodes and above than at one nematode per host in all three assays (extended model; $p_2 > p_1$).

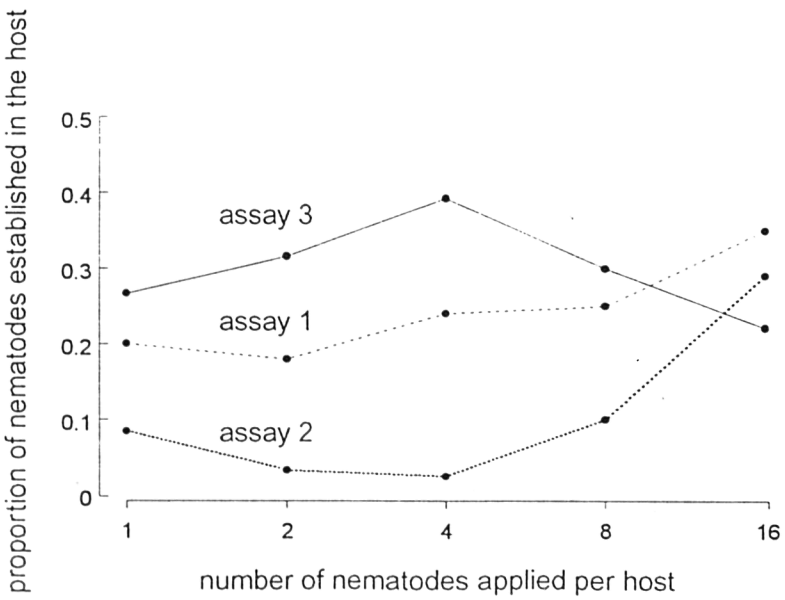

Fig. 1 Proportional establishment of *Hetrorhabditis megidis* in *Galleria mellonella*.

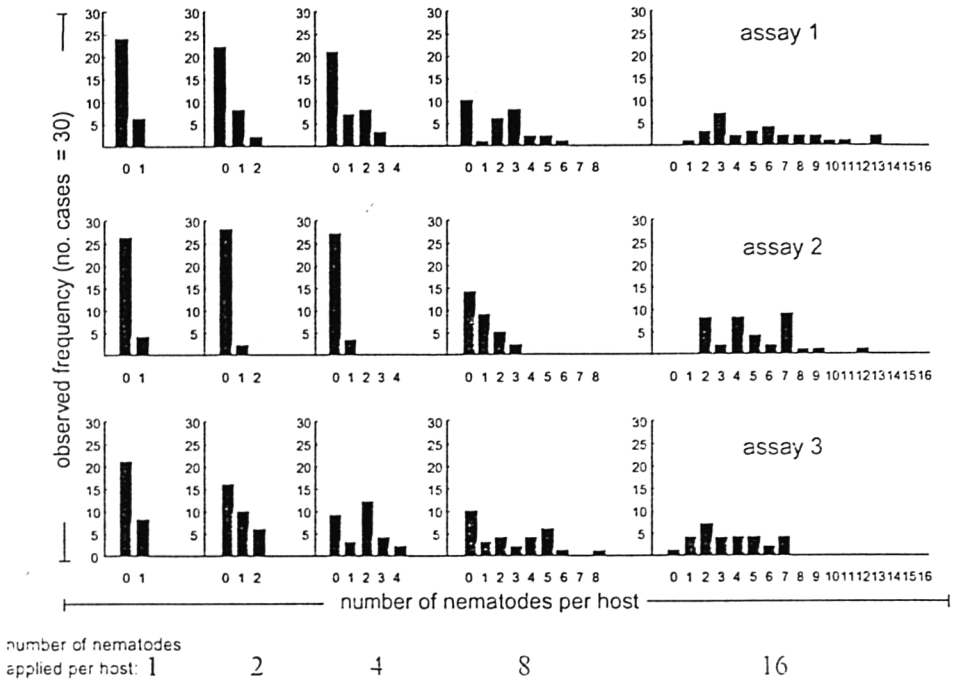

Fig. 2 Frequency distribution of nematode numbers per host.

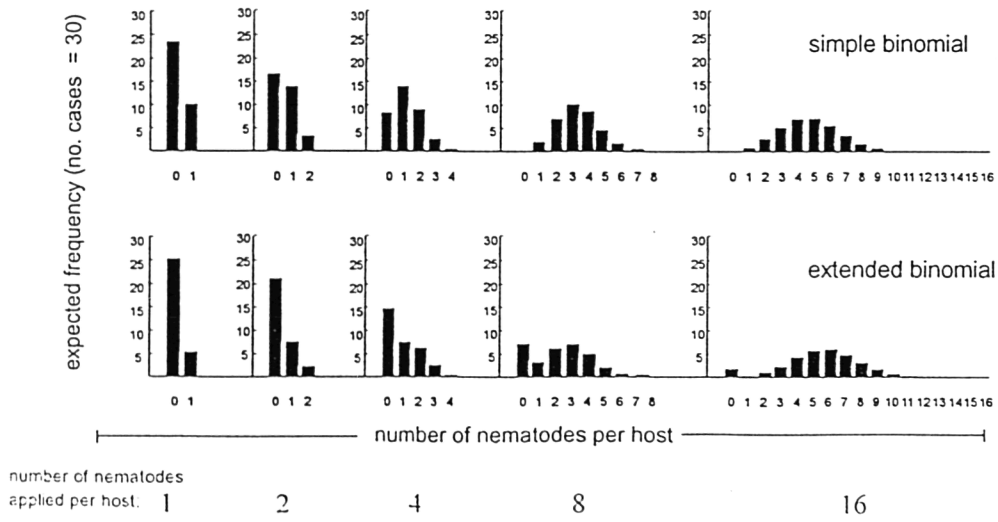

Fig. 3 Expected frequencies for assay 1 using two models of nematode invasion.

The actual numbers of nematodes per host at each dose are shown in Figure 2 and for comparison, Figure 3 shows the expected values that were determined using the simple and extended binomial infection models for data from assay 1. The extended model was statistically superior to the simple binomial in each assay (maximum likelihood ratio test, $P < 0.05$). Thus, the data support the contention that initial infection of a host increases the likelihood of subsequent invasion.

Every infected host died within five days of exposure to nematodes. The wax moth, *G. mellonella* is highly susceptible to entomopathogens (Bedding *et al.,* 1983), and nematode encapsulation rarely occurs in the haemolymph of this host (Dunphy and Webster, 1987). In other insects, however, immune responses can cause significant mortality among invading nematodes. For example, Peters and Ehlers (1994) have reported that 80 - 90% of cranefly larvae (both *Tipula paludosa* and *T. oleracea*), infected with *S. feltiae* caused some, if not all dauer juveniles in the haemocoel to become encapsulated. On average, up to four nematodes could be encapsulated and killed by either *T. paludosa* or *T. oleracea*, but more than four infections generally exceeded the resistance capacity of the host (Peters and Ehlers, 1994). The dynamics of the nematode infection process may therefore be likened to the phenomenon of group attack in bark beetles (Coleoptera: Scolytidae) that infect and kill conifers. Like entomopathogenic nematodes, these insects vector microorganisms that contribute to the pathology of the host and a successful infection can only be established if parasite numbers exceed the host's resistance capacity (Wood, 1972). In this instance, cooperative behaviour is mediated by pheromone aggregation signals (Berryman, 1976) and the likelihood that each individual will survive and reproduce is increased by the activities of conspecific individuals (Berryman *et al.,* 1989). At very high population densities, interference competition can result in density dependent reductions in fecundity, and this too has been documented in entomopathogenic nematodes (Selvan *et al.,* 1993). Dauer juvenile nematodes that emerge from an insect cadaver, do so in large numbers; many of these are likely to recruit into nearby hosts and the likelihood of multiple infection is probably high. Thus group attack may be an important attribute of the nematode/host interaction. This aspect of dauer juvenile behaviour warrants further study. For example, experiments from which the "successful infection threshold" may be determined for individual hosts may provide important data for the biological control of insect pests. The study of opposing rate reactions; insect defence and nematode arrival should be a focus for future research. In particular, a conceptual framework for understanding nematode infectivity and host defence may suggest strategies for control of pest species that are well defended immunologically.

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REFERENCES

Bedding, R.A., Molyneux, A.S. and Akhurst, R.J. (1983). *Heterorhabditis* spp., *Neoaplectana* spp., and *Steinernema kraussei*; interspecific and intraspecific differences in infectivity for insects. Exp. Parasitol. **55,** 249-257.

Berryman, A.A. (1976). Theoretical explanation for mountain pine beetle dynamics in lodgepole pine forests. Environ. Entomol. **5,** 1225-1233.

Berryman, A.A., Raffa, K.F., Millstein, J.A. and Stenseth, N,C. (1989). Interaction dynamics of bark beetle aggregation and conifer defense rates. Oikos **56,** 256-263.

Dunphy, G.B. and Webster, J.M. (1987). Partially characterised components on the epicuticle of dauer juvenile *Sterinernema feltiae* and their influence on haemocyte activity in *Galleria mellonella*. J. Parasitol. **73,** 584-588.

Grewal, P.S., Selvan, S., Lewis, E.E. and Gaugler, R. (1993). Male insect-parasitic nematodes: a colonizing sex. Experientia **49,** 605-608.

Hay, D.B., Fenlon, J.S. and Richardson, P.N. (1994). The analysis of *in vivo* bioassay entomopathogenic nematode research: a behavioural approach. IOBC/WPRS Bull. **17(3),** 136-139.

Hominick, W.M. and Reid, A.P. (1990). Perspectives on entomopathogenic nematology. In: Gaugler, R. and Kaya, H. (Eds.) *Entomopathogenic Nematodes in Biological Control*, pp.327-345. CRC Press, Boca Raton.

Peters, A. and Ehlers, R.-U. (1994). Susceptibility of leatherjackets (*Tipula paludosa* and *Tipula oleracea*) to the entomopathogenic nematode *Steinernema feltiae*. J. Invertebr. Pathol. **63,** 163-171.

Pye, A.E. and Burman, M. (1981). *Neoaplectana carpocapsae*: nematode accumulations on chemical and bacterial gradients. Exp. Parasitol. **51,** 13-20.

Selvan, S., Campbell, J.F. and Gaugler, R. (1993). Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. J. Invertebr. Pathol. **62,** 278-284.

Wood, D.L. (1972). Selection and colonisation of ponderosa pine by bark beetles. In: Van Emden, H.F. (Ed.) *Insect/Plant Relationships*, pp.101-107. Blackwell, Oxford.

INTRA-POPULATION INFECTIOUS STRUCTURE AND TEMPORAL VARIATION IN *STEINERNEMA FELTIAE*

David A. Bohan[1] and William M. Hominick[2]

[1]Imperial College of Science, Technology and Medicine, Silwood Park, Sunninghill, Berkshire, SL5 7PY, U.K. [2]International Institute of Parasitology, 395A Hatfield Road, St Albans, Hertfordshire, AL4 0XU. U.K.

SUMMARY

The authors Bednarek and Nowicki (1986) and Fan and Hominick (1991a) have noted that only a proportion of *Steinernema feltiae* dauer juveniles seem to be capable of causing infection in test hosts at any given time. Both sets of authors alluded to the presence of an infectious proportion, within the dauer population, that may explain this limitation in infection but no evidence was presented. Using experimental, statistical and simple simulation techniques, the presence of an infectious proportion was investigated. The analysis supports the grouping of individual dauers into either infectious or non-infectious proportions and further work suggests that these proportions are dynamic.

INTRODUCTION

The use of a theoretical framework to study parasitic processes has found widespread use in veterinary and medical helminthology. This approach advocates a mathematics-like methodology splitting the interaction being studied into a number of discrete steps which may then be compared with an explicit model for the interaction. The basic unit of study is the individual, either the parasitic stage in the host or the infective stage in the external environment (Anderson, 1982). Using the individual as the unit of study, qualitative and quantitative statistical descriptions of the role of the individual within the population may be achieved.

Little research in entomopathogenic nematology has attempted to study the individual nematode. In addition, it is also rare to find studies that consider the steps that an individual nematode must undergo in completing a process. For example, many infection studies only consider the mortality of a test host, when exposed to a given concentration of dauer juveniles and investigate this infection interaction under the rather contrived conditions of a filter paper or sand tube. Some work, though, has studied the individual nematode. Bednarek and Nowicki (1986) and Fan and Hominick (1991a) both looked at the infection interaction by counting both the numbers of dauer juveniles placed in the test arena and the numbers of parasitic stages (the parasite burden) subsequently observed in the test host. These authors reported that only a proportion of the applied dauers seemed capable of invading and parasitising the test host. It was suggested that this phenomenon may be explained by a proportion of dauers that was infective. However, neither Bednarek and Nowicki (1986) nor Fan and Hominick (1991a) could provide any evidence for the presence of such an infective proportion.

Here we investigate the presence of an infective proportion, which in order to avoid confusion with the term "infective juvenile" we shall call the infectious proportion.

We postulate that the proportion is a structure within the dauer population. The observed data will be tested for dependence against both dauer and host density, temperature and host distribution. The study will also be extended to investigate the reports of Fan and Hominick (1991b). These authors observed that the proportion of dauer stages that became parasitic was very dependent upon the length of storage time, describing what was termed a "U-shaped curve". We shall consider the relationship between the U-shaped curve and the putative infectious proportion. To achieve this, a simple infection model is described against which the experimental data will be tested.

METHODS

Nematode and host cultures.

All *Galleria mellonella* (L) larvae were obtained from a laboratory culture maintained at Silwood Park for over 20 years. The *Steinernema feltiae* (Site 76 strain) was isolated near Newbury, Berkshire in 1987. The *S. feltiae* were cultured using *G. mellonella* (Woodring and Kaya, 1988), at 15°C, and used for experimentation within 6 days of emergence from the culture host.

Experimental arenas.

The experimental arenas were 30 ml universal tubes (Sterilin), filled with 25 ml of silver sand moistened at 4% (v/v) with tap water. 1 ml of an appropriate suspension of *S. feltiae* dauers was dispensed into each tube. Except where otherwise stated, the tubes were then stored at the appropriate experimental temperature overnight (12-15 hours) before use.

For experimentation, up to three *G. mellonella* larvae were introduced into each arena. The tubes were then sealed, inverted and the *G. mellonella* larva "exposed" for up to 72 hours at the experimental temperature. Following exposure, the larvae were removed and incubated for a further 72 hours at 15°C. The insect cadavers were then dissected and the parasite burden each harboured was noted.

Assessment of survival.

Dauer survival was assessed with a sand-wash technique. The contents of a sand arena were dropped into a 250 ml beaker and covered with 25 ml of tap water. The beaker was shaken to suspend the dauers. By pouring off the supernatant the surviving dauers could be counted. Three consecutive washes removed approximately 75-80% of the available dauers (Bohan, unpublished).

Experiments

Expt. 1. Effect of dauer density on infection: The number of parasites establishing within the *G. mellonella*, when exposed to eight standard densities of *S. feltiae* was investigated. Eight suspensions at 100, 200 through to 800 dauers were pipetted into eight sets of ten replicate sand tubes. A single *G. mellonella* was sealed into each tube and exposed at 15°C for 72 hours.

Expt. 2. Effect of host density on infection: 200, 500 or 800 dauer suspensions were pipetted into one of three groups of 30 sand tubes. Within each

group the 30 arenas were divided into sets of ten tubes into which 1, 2 or 3 *G. mellonella* larvae were introduced. The larvae were exposed for 72 hours at 15°C. Thus the parasite burden, observed in each arena, could be studied in terms of the density of dauers and *G. mellonella* within each sand tube.

Expt. 3. Effect of host distribution on infection: The distribution of a host could clearly affect the parasite burden observed during experimentation. In order to study the effect of *G. mellonella* distribution, within a sand arena, two groups of tubes were constructed. 15 tubes were prepared in the normal manner, although the 200 dauers and 3 *G. mellonella* were introduced immediately after construction. For the other group, each tube had one *G. mellonella* larva placed at the base, one at the mid-point and, following immediate introduction of the 200 dauer suspension, one at the top. The larvae were exposed for 72 hours at 15°C.

Expt. 4. Effect of dauer density on parasitic stage accumulation: The host accumulates a parasite burden over time, in a manner that may be dependent upon the density of the parasite population. Eight suspensions at 100, 200 through to 800 *S. feltiae* dauers were pipetted into eight groups of 50 sand tubes. A single *G. mellonella* larva was subsequently placed into each sand arena. Ten tubes were sampled, after 9, 16, 24, 48 and 72 hours of exposure at 15°C, from each host density group and the *G. mellonella* dissected. Thus, a description of the accumulation of parasites was achieved for each *S. feltiae* dauer density.

Expt. 5. Effect of host density on parasitic stage accumulation: A suspension of 200 dauers was pipetted into three groups of 50 sand tubes. Either 1, 2 or 3 *G. mellonella* larvae were introduced into the tubes of each group. The groups were then exposed at 15°C. After 9, 16, 24, 48 and 72 hours, ten arenas were removed from each *G. mellonella* density group. The parasite burden counts for the arenas of each group would provide a profile for the accumulation of parasites and indicate the dependence of parasite accumulation on host density.

Expt. 6. Effect of temperature on parasitic stage accumulation: The ambient temperature must clearly have an effect on the dynamics of a host-parasite interaction. Parasite accumulation was studied at 5, 10, 15, 20, 25 and 30°C. A suspension of 200 dauers was pipetted into six temperature groups of 50 sand tubes. Into each tube, individual *G. mellonella* were placed before the arenas were returned to their respective experimental temperature. Ten tubes were then sampled from each temperature group after exposure periods which reflected the experimental temperature.

Expt. 7. Long-term dynamics of infection: The dynamics of a host-parasite interaction may change over an extended period of time. The number of parasites harboured by *G. mellonella* larvae, following exposure at 15°C, was studied over ten weeks at 15°C. Two hundred and forty sand tubes were prepared, each containing 200 dauers. On day 0 and 1, week 1 and 2, and every subsequent week to week 10, twenty sand arenas were removed. Ten tubes were sampled to assess survival and ten for exposures. The individual *G. mellonella* larvae were exposed for 72 hours.

Expt. 8. Effects of Long-term Dynamics on Parasitic Stage accumulation: The effect of time on the acquisition of parasites was investigated over eight weeks at 15°C. Five hundred and forty arenas were constructed, each

containing 200 *S. feltiae* dauers. On day 0 and every subsequent week to week 8, sixty sand tubes were sampled. Ten tubes were used to assess dauer survival, whilst into the remaining fifty "exposure" arenas individual *G. mellonella* were introduced. Ten tubes were sub-sampled from the exposure arenas at 9, 16, 24, 48 and 72 hours of exposure at 15°C. This produced data for the accumulation of parasitic stages for each week of an 8 week storage period. The 72 hour data-sets could then be directly compared with those of Experiment 7.

Statistics.

The results were analysed using generalised linear modelling methods in the PC package GLIM. Poisson errors were used in conjunction with count data. Proportional data were analysed using the Binomial distribution and logits (log odds). Where the variance of the data were greater than the mean (overdispersion) the data were assumed to conform to the Negative Binomial distribution and an empirical scale parameter (s) was employed to appropriately increase the variance estimates (McCullagh and Nelder, 1989). Changes in Poisson deviance (a measure of discrepancy analogous to variance) were tested against χ^2 tables, whilst for the Binomial distribution F-tests were used. The data were subsequently tested for non-linearity in standardised residuals, normality with standardised probability plots and for leverage (Crawley, 1993).

For the accumulation of parasites, an infection model was used to describe the data. The available data suggest that only a proportion of *S. feltiae* dauers may infect a host at any one given time. This infectious proportion, α, determines the number of dauers capable of infecting a host. Thus if N_0 is the number of dauers applied to an arena, αN_0 is the number of infectious dauers. If the αN_0 infectious dauers randomly contact the L hosts within the arena, with a constant probability per nematode, per host, per unit time of contact and invasion (the transmission coefficient, β) we have an initial number of parasites of $\beta\alpha N_0 Lt$, after time t. However, the number of dauers is finite, so the number of parasites within a host (P_t) rises as;

$$P_{t+d} = \frac{\gamma t}{1 + \beta L t}$$

where $\gamma = \beta\alpha N_0 L$ and d is a time delay, the time taken by the nematodes to penetrate to the haemocoel of the insect. The model was fitted by using a macro, with Poisson errors and the reciprocal link.

The 72 hour parasite burden data for experiments 7 and 8 were analysed as proportion data, using the mean number of surviving dauers as the binomial denominator.

RESULTS

Expt. 1. Effect of dauer density on infection: The parasite burden data were analysed as logits. The density of *S. feltiae* dauers was found to be a non-significant variable ($F_{(1,39)}$=2.81, s=6.97; P>0.05), explaining only 7% of the deviance for parasite establishment. Thus, the proportion of approximately 0.192

dauers becoming parasitic was not dependent upon the density of dauers applied to the sand tubes.

Expt. 2. Effect of host density on infection: The parasite burden data were analysed as logits, using the total number of parasitic stages, from each tube, as the response variable and host density as a co-variate. Parasite burden again proved independent of dauer density ($F_{(1,84)}$=2.31, s=3.763; P>0.05). The co-variate of host density ($F_{(2,84)}$=0.54; P>0.05) and the host-nematode density interaction ($F_{(2,84)}$=0.31; P>0.05) were also non-significant. Thus, despite increasing the dauer and host density the proportion of dauers that become parasitic did not change from 0.321 (Fig. 1).

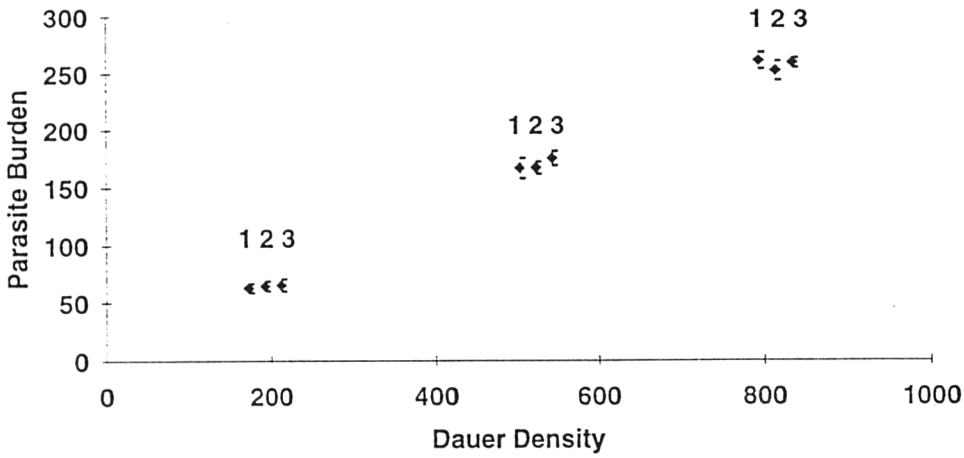


Fig.1. Parasite burdens observed in the different *G. mellonella*-dauer density combinations described in Experiment 2. The data are the mean and the standard error of the mean for the parasite burden. The middle data-point of each density set denotes the dauer density. The superscript numerals denote the *G. mellonella* density.

Expt. 3. Effect of host distribution on infection: The data for the total number of parasitic stages within each arena were analysed as logits against the factor of host distribution. Host distribution was found not to be a significant factor ($F_{(1,27)}$=3.326, s=1.726; P>0.05) in explaining total parasite burden per tube, explaining only 12% of the observed deviance. Approximately 26.8% of the applied dauers became parasitic.

The results of the first three experiments show that changing neither the density of hosts nor dauers, nor changing the distribution of the hosts changes the proportion of dauers that become parasitic. The proportion of dauers becoming parasitic, in any one experiment seems to be fixed. These findings allow us to infer, as a working description, that a population of *S. feltiae* contains a proportion of dauers that are capable of causing parasitism, the infectious proportion.

Expt. 4. Effect of dauer density on parasitic stage accumulation: The data for the accumulation of parasitic stages were analysed using the infection model. At all densities, the infection model was a significant fit to the data (Table 1).

TABLE 1. Analysis for the Infection Model, fitted to data collected at different *S.feltiae* dauer densities.

	Dauer Density							
	100	200	300	400	500	600	700	800
Model χ^2	χ_2^2= 63.72	χ_2^2=96.56	χ_1^2=25.78	χ_1^2=48.88	χ_1^2=21.69	χ_1^2=39.42	χ_1^2=49.02	χ_1^2=122.7
Probability	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Time Delay χ^2	χ_1^2=7.21	χ_1^2=4.10	*	χ_1^2=0.16	χ_1^2=0.12	χ_1^2=0.001	χ_1^2=0.15	χ_1^2=1.67
Probability	P<0.01	P<0.05	-	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Proportion of Deviance Explained	0.77	0.81	0.52	0.68	0.48	0.62	0.67	0.84
Scale Parameter "s"	1	2.45	3.73	4.38	4.89	6.46	6.12	5.02

* minimum deviance for a time delay of zero hours

The infectious proportion was estimated to be independent of the density of dauers applied to the arena, a finding that is compatible with the earlier experiments. The transmission coefficient β was found to decline with an increase in dauer density, in an almost reciprocal manner. This observation was, though, inconsistent with the model for which the assumption was made that the transmission coefficient was a constant. Simulation studies showed that this reciprocal relationship between the transmission coefficient and the density of dauers was either a biological property or a property of the sand tube system, and not a pathological consequence of the model fitting process.

Expt. 5. Effect of host density on parasitic stage accumulation: The data for the accumulation of parasites for each arena were analysed using the infection model. The model fitted the data highly significantly for all host densities. The infectious proportion was estimated to be independent of host density, as in earlier experiments, at 0.367 (S.E. +0.051 -0.040), 0.347 (S.E. +0.031 -0.0263) and 0.365 (S.E. +0.030 -0.026) for the 1, 2 and 3 host density groups respectively. As expected the probability of becoming parasitic, per unit time, increased with host density although the transmission coefficient declined. The transmission coefficient was assumed to be constant with changes in host density. Again simple simulation studies showed the observed decrease in the transmission coefficient to be either a biological property or a property of the sand tube system.

Expt. 6. Effect of temperature on parasitic stage accumulation: The parasite burden accumulation data were analysed using the infection model. The model was found to fit the data highly significantly at all temperatures except 30°C. 30°C is close to the upper limit of *S. feltiae* temperature tolerance (Yang and Li, 1988) and consequently nematode behaviour at this temperature was not considered characteristic. The transmission coefficient rose from 0.0068 (S.E. +0.0012 -0.0009) at 5°C, to 0.052 (S.E. +0.0056 - 0.0046) at 20°C, but fell to 0.033 (S.E. +0.0041 -0.0033) at 25°C, indicating that the probability of invasion per unit time is temperature dependent. Despite changes in the transmission coefficient, the estimated infectious proportion remained remarkably constant at all temperatures (Fig. 2).

For experiments 4-6, the behaviour of the model-estimated infectious proportion was remarkably similar to the number of dauers that became parasitic in the first three experiments. The estimated infectious proportion did not change with changes in the size of the dauer or host population, nor did the proportion change with temperature when changes in the transmission coefficient were observed. We suggest that this similarity, following statistical and model analyses, would confirm the presence of the infectious proportion.

This argument is also independent of the departure of the model-estimated transmission coefficient from expectation, for the assumptions of this parameter. The declines in the transmission coefficient, with host and dauer density, may simply be explained by non-random host and dauer contact and invasion, contrary to the model assumptions. This non-random contact and invasion may be due to a biological property or due to a property of the sand tube system as suggested by the simulation studies. Such non-randomness may be accounted for using the scale parameter, thus ensuring a valid estimation of the infectious proportion, and does not invalidate the model.

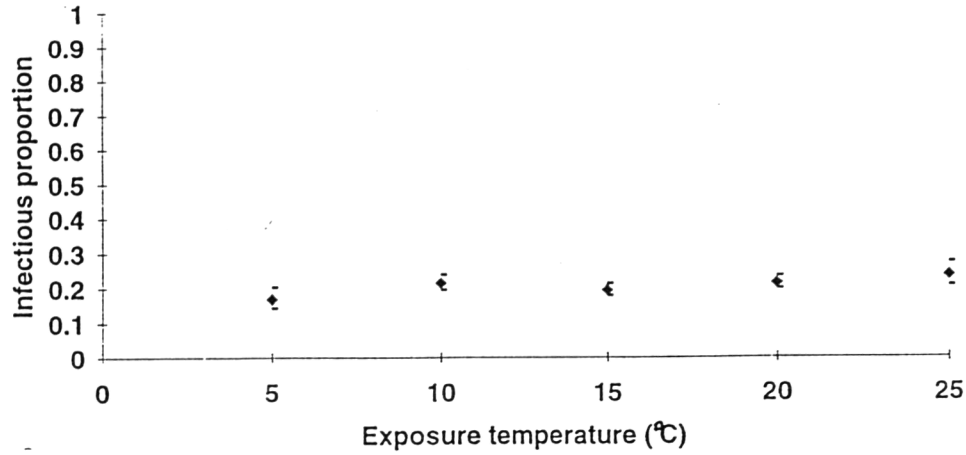


Fig 2. Infectious proportion at each temperature, estimated by the infection model. The data are the estimated mean and the standard error of the mean for the infectious proportion.

Expt. 7. Long-term dynamics of infection: The number of dauers extracted from the sand tubes initially declined steeply, from 146.6 (S.E.=±3.0) dauers on day 0 to 108.3 (S.E.=±5.3) dauers on day 14. The numbers then declined much more slowly, over the rest of the experimental period, to about 95 (S.E.=±6.5) dauers on day 70.

The number of dauers that became parasitic fluctuated over the course of the experiment. The number of parasitic stages were analysed as logits, with the number of sand-wash extracted dauers as the Binomial denominator. Logit analysis of the data showed that the proportion of establishing dauers fluctuated significantly over the ten week course of the experiment ($F_{(10,98)}$= 5.64, s=8.0; P<0.001, Fig. 3).

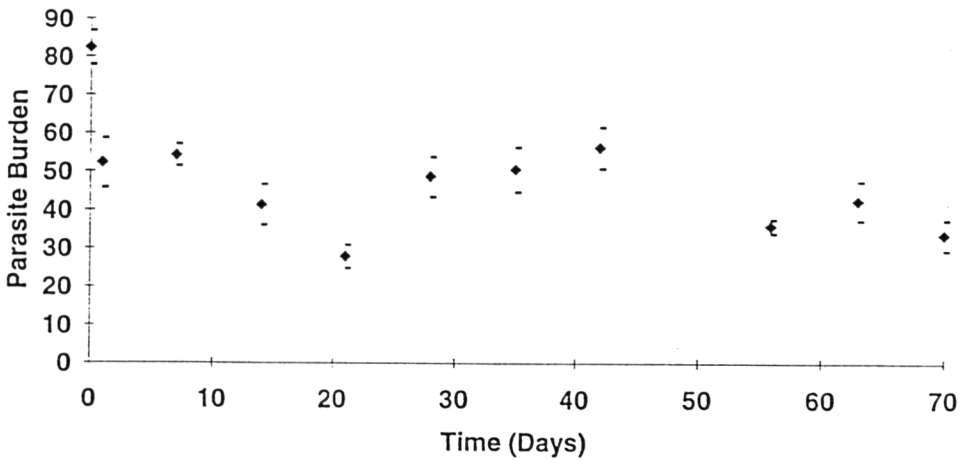


Fig. 3. The observed parasite burden over a ten week time course. The data represent the mean and the standard error of the mean number of dauers becoming parasitic for each sample date.

Expt. 8. Effects of long-term dynamics on parasitic stage accumulation: The number of dauers extracted using the sand wash technique declined in a very similar manner to that of the earlier experiment, from 145.3 (S.E.=±5.0) individuals on day 0 to about 70.5 (S.E.=±2.8) dauers in week 8.

The infection data gathered by considering the 72 hour data-sets were analysed as logits. The data for the number of dauers that became parasitic showed significant changes over the course of the experiment ($F_{(8,81)}$=7.61, s=7.0; P<0.001), rather as in the previous experiment.

The changes in the numbers of dauers becoming parasitic, over time, were investigated by fitting the infection model to the cumulative data-sets, for each sampling day. The model fitted the data significantly on all sampling days. The total number of infectious dauers (the infectious population), estimated by the model for all sampling days, was found to be directly related to the number of dauers that became parasitic in the *G. mellonella* after 72 hours in that week (Fig. 4.). The analysis suggests that the changes in the magnitude of the observed parasite burden, during the experiment, are at least in part due to changes in the size of the infectious proportion. The estimates for the transmission coefficients fluctuated over the course of the experimental period, although with little trend, and no dependence between the transmission coefficient and the estimated infectious proportion was observed.

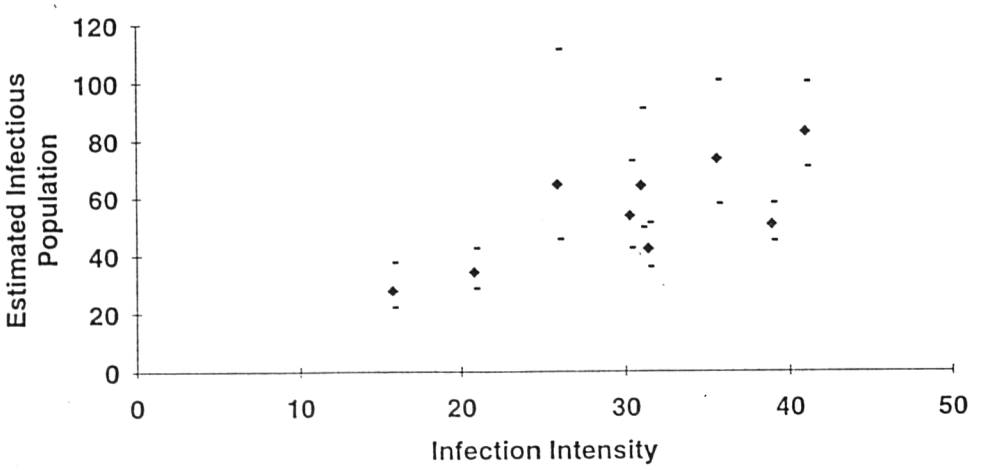


Fig. 4. The relationship between the infectious population, estimated using the infection model, and the infection intensity observed in the experiment. The data are the estimated mean and standard error of the mean for the infectious population against the mean observed parasite burden.

The fluctuations in the observed number of dauers that become parasitic and in the estimated infectious proportion may explain why the magnitude of the infectious proportion appears constant within an experiment, yet is clearly different between experiments.

DISCUSSION

This communication shows that populations of *S. feltiae* contain an infectious proportion, that limits infection in the laboratory. The presence of this proportion was determined using a static ANOVA approach and a dynamical approach with a simple infection model, based upon an assumption of the presence of an infectious proportion, fitted to empirical data. The infectious proportion is not an artefact of the sand tube system, but is stable and present despite changes in the density of hosts and dauers or changes in temperature or host distribution. The analysis also makes clear some of the behaviour of the sand tube system. The infectious dauers, those dauers that belong to the infectious proportion, may be assumed to be in direct contact with a host within a sand arena. This is because changing host distribution does not change the proportion of dauers that become parasitic. In essence the hosts may be placed anywhere within the tubes and the same results will be achieved. Consequently, the sand tubes may be considered to have no space as such and the dauers share the same space with the host or hosts.

The infection model enforces a change in emphasis in the manner the *S. feltiae-G. mellonella* interaction is perceived. The infectious dauers that are described by the model have a constant infection probability, the transmission coefficient. The infectious dauers are also in contact with the host. Thus the model simply describes the host penetration phase of the process of host parasitism. Following the definitions of Pike (1990), an "infective stage" must perform a number of behaviour patterns in order to gain access to a host. First, the stage performs behaviour patterns designed to maximise contact with the host, such as moving into host space. The stage must then migrate towards the host, following its detection. Finally, the infective stage is in contact with the host and must undergo penetration behaviour patterns. Whereas previously the dauers may have been described as undergoing all three infection behaviours in the sand tube, in this scheme, the model is describing the final phase, the act of host penetration.

The use of the model demonstrated that the proportion of dauers that became parasitic and the estimated infectious proportion were directly related. However, the analysis failed to explain why the infectious proportion changed between experiments. Was it due to different cultures? While different cultures certainly play a role, Fan and Hominick (1991b) have shown that the proportion of dauers that became parasitic fluctuates over the course of an experiment, a phenomenon the authors termed the "U-shaped curve". Using the infection model, this study demonstrates that the infectious proportion does indeed fluctuate, in a manner very similar to the U-shaped curve of Fan and Hominick (1991b), over the course of 8 to 10 weeks. Presumably, this fluctuation explains the majority of the change in the infectious proportion observed between experiments.

The fluctuation in the magnitude of the infectious proportion would indicate that while stable, the infectious proportion is not static but dynamic. Clearly there are changes in the make-up of the infectious proportion such that it may fluctuate over time. At present, the mechanism the controls the fluctuation is unknown, but it may include both maturation effects or exchange between the infectious and a "non-infectious" proportion. Bohan (unpublished), though, has observed that previous exposure to a host does not result in infectiousness.

The presence of an infectious proportion which, although constant within experiments, changes over the course of time has marked implications for the correct construction of bioassays. This work has shown that each individual dauer is different, having its own particular infectious status and having a particular infectivity (a variable related to the transmission coefficient). Thus, the use of prevalence studies, which assume that all dauers are similar and which only quantify the number of exposed hosts which become parasitised, might be described as flawed as such assays miss much of the biology of the interaction. In turn, the use of single dauers for the assessment of infectivity is also not advisable. While each dauer is undoubtedly different, and should be studied as such, the dauers occur within a context of other individuals. It is the belief of the authors that the fluctuations present in the infectious proportion are controlled at the level of the population. Consequently, to use a single dauer for experimentation is to remove it from the population context; a situation that is likely to modify the infection behaviour of the dauer.

The ecological significance of the infectious proportion and the U-shaped curve are more difficult to fathom. It has been noted by Fan and Hominick (1991a) and Hominick and Reid (1990) that limiting the numbers of dauers capable of infection, at any one time, would have benefits in terms of reducing potentially massive levels of parasitism. Such massive parasite burdens would occur where a host comes into contact with large numbers of dauers, and would lead to markedly reduced fitness, both in the stages undergoing infection and in their progeny. However, it is the belief of the authors that the probability of contact between a host and a dauer, in the field, is so low that the effect of an infectious proportion would be to prevent parasitism, reducing dauer fitness further. Here we shall "stick out our necks" and consider a somewhat more radical view of the significance of the infectious proportion.

Populations of dauers exist as aggregated groups within the environment, the groups being spatially structured according to the point at which their host cadaver came to rest. Assuming low intra-population genetic diversity, steinernematid populations are highly related to their parental populations. Infected hosts are unlikely to travel far, so locally distributed populations are also likely to be quite closely related. Thus within a locality the spatially structured populations of dauers are likely to share a similar genotype. Given this, a mechanism for producing a-synchrony in behaviour may increase the time to extinction (Harrison and Quinn 1989; Hochberg, 1989). In simple terms, the fluctuations in the infectious proportion occurring a-synchronously within each dauer population may serve to increase the time to extinction of the genotype.

REFERENCES

Anderson, R.M. (1982). Epidemiology. In: Cox, F.E.G. (Ed.), *Modern Parasitology*, pp. 204-251. Blackwell Scientific Publications, Oxford.

Bednarek, A. and Nowicki, T. (1986). Effect of intrapopulation factors in the nematodes *Steinernema feltiae* (Steinernematidae) on intensity of insect infestation. Zeszyty Problemowe Postepów Nauk Rolniczych, **323**, 199-212.

Crawley, M.J. (1993). *GLIM for Ecologists*. Blackwell Scientific Publications, Oxford.

Fan, X. and Hominick, W.M. (1991a). Efficiency of the *Galleria* (wax moth) baiting technique for recovering infective stages of entomopathogenic rhabditids (Steinernematidae and Heterorhabditidae) from sand and soil. Rev. Nematol. **14**, 381-387.

Fan, X. and Hominick, W.M. (1991b). Effects of low storage temperature on survival and infectivity of two *Steinernema* species (Nematoda: Steinernematidae). Rev. Nématol. **14**, 407-412.

Harrison, S. and Quinn, J.F. (1989). Correlated environments and the persistence of metapopulations. OIKOS **56**, 293-298.

Hochberg, M.E. (1989). The potential role of pathogens in biological control. Nature, **337**, 262-265.

Hominick, W.M. and Reid, A.P. (1990). Perspectives on entomopathogenic nematology. In: Gaugler, R. and Kaya, H.K. (Eds.) *Entomopathogenic Nematodes in Biological Control*, pp. 327-345. CRC Press, Boca Raton.

McCullagh, P. and Nelder, J.A. (1989). *Generalized Linear Models.* Chapman and Hall, London.

Pike, A.W. (1990). Interpreting parasite host location behaviour. Parasitology Today **6**, 343-344.

Woodring, J.L. and Kaya, H.K. (1988). *Steinernematid and Heterorhabditid Nematodes: A Handbook of Techniques.* Southern Cooperative Series Bulletin 331, Fayetteville, Arkansas.

Yang, P. and Li, S.C. (1988). The effect of temperature on the development and pathogenicity of entomopathogenic nematodes. Entomol. Knowl. **25**, 300-302.

PREDICTORS OF FORAGING STRATEGY IN ENTOMOPATHOGENIC NEMATODES

Parwinder S. Grewal[1], Randy Gaugler[2] and Ramon Georgis[1]

[1]biosys, 1057 East Meadow Circle, Palo Alto, CA 94303, USA.

[2]Department of Entomology, Rutgers University, New Brunswick, NJ 08903, USA.

SUMMARY

Description of new species of entomopathogenic nematodes has escalated tremendously in recent years. Most of these species are collected from soil with almost no knowledge about their natural host associations. Since ecological and behavioural barriers severely restrict actual host ranges of these parasites, it is imperative that information about their foraging behaviour is obtained prior to extensive field tests. We propose that nematode foraging strategy may be predicted from their behavioural repertoire. Nictation behaviour and response to long range chemical cues serve as two important predictors of foraging strategy. Positive directional response to chemical cues and absence of nictation behaviour in *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema anomali*, and *S. glaseri* relate to their ability to parasitize distant subterranean insects. The absence of directional response to host volatiles and presence of nictation behaviour in *S. carpocapsae* and *S. scapterisci* relate to their ability to parasitize highly mobile surface dwelling insects. These two categories represent two extreme modes of foraging: ambushing and cruising. *S. feltiae* and *S. riobravis* are intermediary in the search continuum sharing some characteristics of both ambush and cruise foragers. *S. feltiae* and *S. riobravis* respond directionally to host volatiles, do not nictate, and parasitize both mobile and sedentary insects effectively.

INTRODUCTION

Foraging strategy is a trait shaped by natural selection, and is manifested in an animals' behavioural repertoire (Hassel and Southwood, 1978; O'Brian *et al.*, 1990). Search behaviour of predators and parasites is a continuum that can be categorized into two extreme modes of foraging: cruising and ambushing. These categories have also been called 'widely ranging' and 'sit-and-wait' (Huey and Pianka, 1981). In cruise search, the forager moves continuously through the environment, searching constantly for prey. In ambush search, a forager remains stationary for long periods of time, waiting for the prey. Long range chemical cues are heavily used by cruisers for locating resources, but such cues are relatively unimportant for the ambush foragers (Bell, 1991). Ambushers search temporally, and are thus most effective at locating highly mobile, densely-spaced prey. Cruisers search spatially and are most effective at locating relatively sedentary, sparsely distributed hosts (Huey and Pianka, 1981; Bell, 1991).

Entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) are soil-inhabiting insect parasites that possess potential for biological control

(Gaugler and Kaya, 1990; Kaya and Gaugler, 1993). The only free-living stage is the infective third-stage juvenile that locates hosts and initiates the parasitic cycle. Steinernematids are mutualistically associated with *Xenorhabdus* spp. bacteria whereas heterorhabditids are associated with *Photorhabdus* spp. (Boemare *et al.,* 1993). After gaining access to the host haemocoel, the bacteria multiply, killing the host within 24-48 h, and create conditions suitable for the development and reproduction of nematode parasitic stages. When host nutrients are depleted, infective stages are produced facultatively that emerge from the cadaver and initiate host search.

Entomopathogenic nematodes have a broad laboratory host range (Poinar, 1990). However, ecological and behavioural barriers restrict their natural host range (Gaugler, 1988). Numerous field tests conducted over a three decade period have revealed that *Steinernema carpocapsae* is more effective at parasitizing surface dwelling insects, whereas *Steinernema glaseri* is generally more effective at killing subterranean insects such as *Popillia japonica* (Kaya, 1990; Georgis and Gaugler, 1991; Kaya and Gaugler, 1993). Such experiments suggested that entomopathogenic nematodes may utilize different foraging strategies. As more and more new species of entomopathogenic nematodes are discovered, it is imperative that some knowledge of their foraging behaviour is obtained prior to field efficacy tests. Following characteristics of entomopathogenic nematodes may be used to predict their foraging strategy.

RESPONSE TO HOST CHEMICAL CUES

Entomopathogenic nematode species differ in their response to host chemical cues. Laboratory studies of the behaviour of *S. carpocapsae* indicate a minimal response to hosts. Only 4% of infective juvenile nematodes responded to volatiles produced by *Galleria mellonella* larvae within 1 h and most never left the inoculation zone (Gaugler *et al.,* 1990). Schmidt and All (1978; 1979), using a similar bioassay technique to that of Gaugler *et al.* (1989), suggested that *S. carpocapsae* infective juveniles were attracted to various host associated materials over 24 h. However, the observation of an aggregation after 24 h exposure to a stimulus lacks the resolution to discern attraction from arrestment (Kennedy, 1978), and the target of attraction was only 0.5 cm from the inoculation zone.

Grewal and Wright (1992) developed a quadrant plate bioassay in which directionality of nematode response to a stimulus could be studied. Using this assay, mean distance travelled per nematode towards or away from the hosts could be determined within 5-30 min after inoculation. We found that infective juveniles of *H. bacteriophora*, *H. megidis*, *S. anomali*, *S. feltiae*, *S. glaseri* and *S. riobravis* directionally respond to host volatiles, whereas *S. carpocapsae* and *S. scapterisci* do not (Grewal *et al.,* 1994) (Fig. 1).

Similar patterns could be discerned while studying nematode responses to contact cues. In an unrewarded search, *S. glaseri* altered their search path from long range to localized search after contact with cuticle or faeces from potential hosts, but *S. carpocapsae* did not (Lewis *et al.,* 1992). Grewal *et al.* (1993) reported that *H. bacteriophora* and *S. glaseri* changed their scanning behaviour during contact with faeces of potential hosts, but *S. carpocapsae* did not. Changes in the scanning behaviour included reduced duration of forward crawling and increased

frequency and duration of backward crawling, stopping, body waving, head waving, head rubbing, and head thrusting.

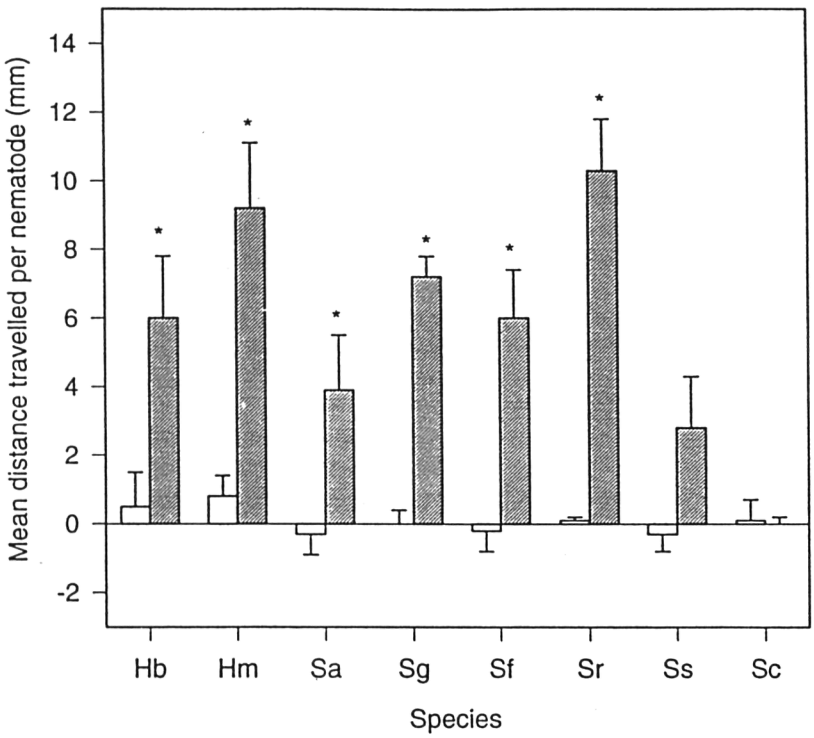


Fig. 1. Response of nematodes to host volatile cues: mean distance (±SE) travelled per infective juvenile on control (no host) (□) and on treated (with host volatiles) (▨) quadrant plates 30 min after inoculation. Nematodes are Hb = *Heterorhabditis bacteriophora*, Hm = *H. megidis*, Sa = *Steinernema anomali*, Sg = *S. glaseri*, Sf = *S. feltiae*, Sr = *S. riobravis*., Ss = *S. scapterisci*, and Sc = *S. carpocapsae*. *Values for the same species with asterisk are significantly different at $P<0.05$ (t-test). (Modified from Grewal *et al.* ,1994).

It is not implied that *S. carpocapsae* never responds to chemical host cues. Infective juvenile *S. carpocapsae* were repelled from cockroach faeces (Grewal *et al.*, 1993) and hosts with heterospecific and heterogeneric infections (Grewal *et al.*, 1995). Lewis *et al.* (1995) reported that *S. carpocapsae* responds to volatiles only after contact with host cuticle. These differences in nematode responses to chemical cues reflect differences in their foraging strategy. Chemical cues are heavily used by cruisers for locating resources in many animals, but such cues are relatively unimportant for the ambush foragers (Bell, 1991).

NICTATION BEHAVIOUR

Nictation occurs when a nematode lifts all but its posterior tip from the substrate and waves the body in three-dimensional spirals and loops (Reed and Wallace, 1965;

Croll and Methews, 1977; Ishibashi and Kondo, 1990). *S. carpocapsae* and *S. scapterisci* nictate (Reed and Wallace, 1965; Kondo and Ishibashi, 1986; Campbell and Gaugler, 1993), whereas *S. anomali*, *S. glaseri*, *H. bacteriophora*, and *H. megidis* do not (Campbell and Gaugler, 1993; Grewal *et al.*, 1994). *S. feltiae* has been reported to nictate but only for short periods of time (Kondo and Ishibashi, 1986), but the described behaviour more closely resembles "body-waving" (*sensu* Campbell and Gaugler, 1993).

Since nictation is a relatively stationary tactic, the nictating species should travel shorter distances on substrates allowing nictation. Nematodes are unable to nictate on smooth substrates such as an agar surface. However, nematodes were found to nictate on agar overlaid with small sand grains (<100 µm). This has two main advantages: (i) it allows microscopic examination of nematodes while performing behaviours, and (ii), it provides some of the complexities of soil. Grewal *et al.* (1994) found that the majority of *S. carpocapsae* and *S. scapterisci* infective juveniles became stationary almost immediately after they were released on the rough substrate. Therefore, both *S. carpocapsae* and *S. scapterisci* travelled significantly less on the rough than on the smooth substrate (Fig. 2). In contrast, *H. bacteriophora*, *H. megidis*, *S. anomali*, and *S. glaseri* travelled equal distances on smooth and rough substrates. Campbell and Gaugler (1993) quantified the time budgeted by individual infective juveniles to nictation on rough substrates. They reported that *H. bacteriophora*, *S. feltiae*, and *S. glaseri* spent more time crawling, and therefore, travelled farther and searched larger areas than *S. carpocapsae* and *S. scapterisci* which travelled shorter distances and searched smaller areas because they spent most of the observation period nictating.

Although *S. feltiae* and *S. riobravis* do not nictate, they still travelled significantly lesser distances on rough than on smooth substrate (Grewal *et al.*, 1994). Both these species indulge in a behaviour called body waving (*sensu* Campbell and Gaugler, 1993), in which the nematode displaces from 30-95% of its body and move back and forth. This behaviour is equivalent to "bridging" (Reed and Wallace, 1965) and "large waving" (Ishibashi and Kondo, 1990).

Nictation may be used by nematodes as an ambushing search tactic. Campbell and Gaugler (1993) reported that nictating *S. carpocapsae* was up to 43 times as effective at finding mobile insect hosts compared to a non-nictating species, *H. bacteriophora*. They suggested that nictation, by reducing the surface tension forces holding the nematode to the substrate, can increase the nematodes' ability to attach to passing insects. The differences in dispersal behaviour on smooth or rough substrates and the presence or absence of nictation behaviour among nematode species predict differences in their search strategies.

HOST PARASITISM ON A SURFACE OR IN A THREE-DIMENSIONAL MATRIX

Another predictor of nematode foraging strategy is their differential ability to forage along the surface or in a 3-dimensional matrix. Grewal *et al.* (1994) reported that infective juvenile *H. bacteriophora*, *H. megidis*, *S. anomali*, and *S. glaseri* were more effective at establishing in hosts in sand columns, but *S. carpocapsae* and *S. scapterisci* were more effective on filter papers (Fig. 3). For instance, about 44% of infective juvenile *S. carpocapsae* located and established in hosts on filter papers, but less than 3% did so in the sand columns. By contrast, 29% of *H. bacteriophora*

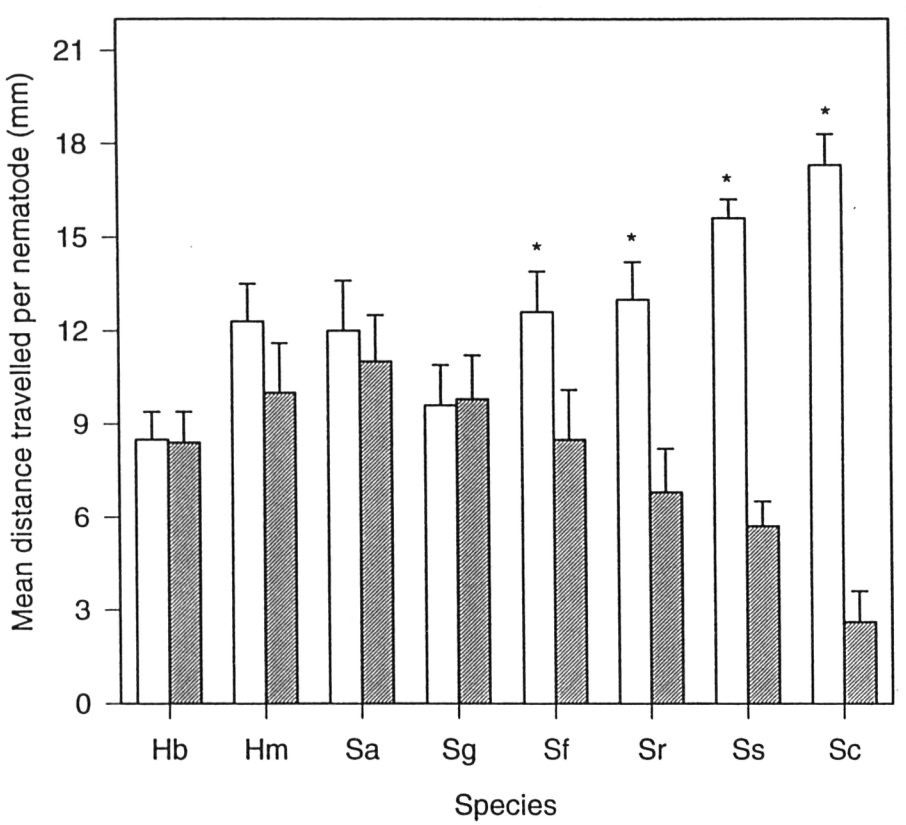


Fig. 2. Effect of substrate on dispersal of nematodes: mean distance (±SE) traveled per infective juvenile on smooth (agar) (□) and rough (agar overlaid with sand grains) (▨) substrate 30 min after inoculation. Nematodes are Hb = *Heterorhabditis bacteriophora*, Hm = *H. megidis*, Sa = *Steinernema anomali*, Sg = *S. glaseri*, Sf = *S. feltiae*, Sr = *S. riobravis.*, Ss = *S. scapterisci*, and Sc = *S. carpocapsae*. *Values for the same species with asterisk are significantly different at $P<0.05$ (t-test). (Modified from Grewal *et al.,* 1994).

infective juveniles established in hosts in sand columns, but only 4% did so on filter papers. *S. riobravis* and *S. feltiae* were equally effective in the two arenas.

Only species utilizing nictation as a host-finding tactic tend to search on the surface (Campbell and Gaugler, 1993). Alatorre-Rosas and Kaya (1990) found that *S. carpocapsae* was more effective at infecting hosts near the soil surface. In addition, Moyle and Kaya (1981) and Georgis and Poinar (1983a) reported a tendency of *S. carpocapsae* to remain near the soil surface. There have also been anecdotal reports of *S. carpocapsae* tendency to remain actually on the soil surface (Reed and Carne, 1967; Kondo and Ishibashi, 1986; Epsky *et al.,* 1988).

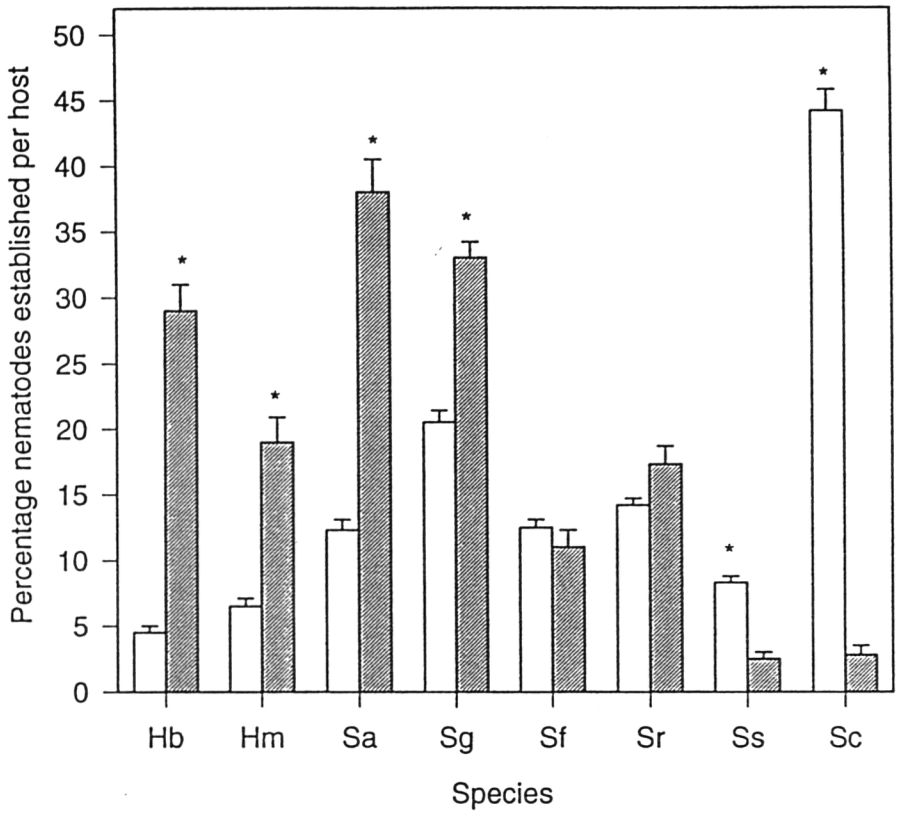


Fig. 3. Proportion (mean±SE) of infective juvenile nematodes established per host when exposed on filter paper (□) or, in sand column (▨). Nematodes are Hb = *Heterorhabditis bacteriophora*, Hm = *H. megidis*, Sa = *Steinernema anomali*, Sg = *S. glaseri*, Sf = *S. feltiae*, Sr = *S. riobravis*., Ss = *S. scapterisci*, and Sc = *S. carpocapsae*. *Values for the same species with asterisk are significantly different at $P<0.05$ (t-test). (Modified from Grewal *et al.*, 1994).

DISCUSSION

Infective juvenile *H. bacteriophora*, *H. megidis*, *S. anomali*, and *S. glaseri* do not nictate, but respond directionally to host cues. Such characteristics represent the behavioural repertoire of cruisers which rely heavily on chemical cues for locating their prey, and search continuously through the environment (O'Brian *et al.*, 1990; Bell, 1991). The absence of directional response to long range host volatiles by *S. carpocapsae* and *S. scapterisci*, ability to nictate, and reduction in dispersal rates on the rough substrates is predictive of an ambushing mode of foraging.

Steinernema feltiae and *S. riobravis* possess some characteristics of ambush and some of cruise foragers (Grewal *et al.*, 1994). Directional response to host cues and inability to nictate are characteristics shared with cruise foragers, whereas less distance traveled on a rough substrate is a trait shared with ambush foragers. *S.*

feltiae and *S. riobravis* can locate hosts effectively on two-dimensional rough substrates and in three-dimensional matrices. Campbell and Gaugler (unpublished data) found that *S. feltiae* is equally effective at finding mobile and non-mobile hosts on a two-dimensional nictation substrate. Although *S. feltiae* and *S. riobravis* do not show nictation behaviour (*sensu* Campbell and Gaugler), a proportion of infective juveniles were seen 'body-waving' on sand grains. Body-waving has been described as lifting one-third to two-thirds of the anterior of the body from the substrate with side-to-side movements (Campbell and Gaugler, 1993; Grewal *et al.,* 1993). We propose that *S. feltiae* and *S. riobravis* may utilize body waving to attach to mobile hosts on a rough substrate.

Steinernema feltiae appears to be a jack-of-all-foraging strategest and a master-of-none. Although infective juvenile *S. feltiae* responded directionally to host volatile cues, they did not aggregate beneath the hosts (Grewal *et al.,* 1994). Using the same assay procedure, Lewis *et al.* (1994) found that *S. feltiae* infective juveniles aggregate beneath the hosts only after they were exposed to contact with host cuticle. This is a trait shared with ambushing nematode species, such as *S. carpocapsae* which respond to host volatiles directionally after contact with host cuticle. The absence of nictation, however, distinguishes *S. feltiae* from the ambusher species.

Foraging strategies of entomopathogenic nematodes can be arranged along a continuum based on the relative success of infective juveniles at finding hosts on or near the surface versus distant hosts in sand columns (Grewal *et al.,* 1994). *Heterorhabditis bacteriophora*, *H. megidis*, *S. anomali*, and *S. glaseri* were most effective at finding distant hosts in the sand columns, thus representing the cruising extreme. Alatorre-Rosas and Kaya (1990) reported that *H. bacteriophora* infected hosts as far as 35 cm vertically and 30 cm horizontally. Of the three steinernematids, *S. glaseri* possessed the greatest dispersal ability (Georgis and Poinar, 1983b; Schroeder and Beavers, 1987; Alatorre-Rosas and Kaya, 1990), and *S. carpocapsae* the least (Molye and Kaya, 1981; Georgis and Poinar, 1983a; Schroeder and Beavers, 1987; Gaugler *et al.,* 1990; Gaugler and Campbell, 1991). *Steinernema carpocapsae* and *S. scapterisci* are most effective at finding hosts on filter paper and perform poorly in the sand column, thus representing the ambushing extreme. *Steinernema feltiae* and *S. riobravis* were equally effective at locating hosts on filter paper and in the sand column (Grewal *et al.,* 1994). Therefore, *S. feltiae* and *S. riobravis* are intermediary between the two extreme modes of foraging. Alatorre-Rosas and Kaya (1990) showed that *S. feltiae* was intermediate between *S. glaseri* and *S. carpocapsae* at finding distant hosts.

As ambushers search on the surface where nictation can occur, and cruisers search for subterranean hosts, these foraging strategies may result in host specialization among entomopathogenic nematodes. Kaya *et al.* (1993) demonstrated that, in the same habitat, *S. carpocapsae* more effectively parasitized the surface dwelling larvae of the black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) and *H. bacteriophora* was more effective at killing the black vine weevil *Ottiorhynchus sulcatus* (Coleoptera: Curculionidae). Isolates of *H. bacteriophora*, *H. megidis*, *S. anomali*, and *S. glaseri* are often found associated with subterranean scarab larvae (Poinar, 1979; 1990). Isolates of *S. carpocapsae* and *S. scapterisci* generally have been found associated with surface-dwelling lepidopteran and orthopteroid insects, respectively. *Steinernema feltiae*, however,

has been isolated from both subterranean hosts such as black vine weevil and from surface-dwelling lepidopteran hosts, e.g., *Heliothis armigera* (Lepidoptera: Noctuidae). Differences among spatial search ranges may reduce competition in these parasites that otherwise possess extremely broad laboratory host ranges.

REFERENCES

Alatorre-Rosas, R. and Kaya, H. K. (1990). Interspecific competition between entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* for an insect host in sand. J. Invertebr. Pathol. **55**, 179-88.

Bell, W. J. (1991). *Searching Behaviour: The Behavioural Ecology of Finding Resources*. Chapman and Hall, London.

Boemare, N. E., Akhurst, R. J. and Mourant, R. G. (1993). DNA relatedness between *Xenorhabdus* spp. (Enterobacteriacae) symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus lumniscens* to a new genus, *Photorhabdus* gen. nov. Intl. Syst. Bacteriol. **43**, 249-255.

Campbell, J. F. and Gaugler, R. (1993). Nictation behaviour and its ecological implications in entomopathogenic nematode search strategies. Behaviour **126**, 155-169.

Croll, N. A. and Mathews, B. E. (1977). *Biology of Nematodes*. John Wiley and Sons, New York.

Epsky, N. D., Walter, D. E., and Capinera, J. L. (1988). Potential role of nematophagous microarthropods as biotic mortality factors of entomopathogenous nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). J. Econ. Entomol. **81**, 821-825.

Gaugler, R. (1988). Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agric., Ecosys. Environ. **24**, 351-360.

Gaugler, R., Campbell J. F. and McGuire, T. R. (1990). Selection for enhanced host-finding in *Steinernema feltiae*. J. Invertebr. Pathol. **54**, 363-72.

Gaugler, R. and Kaya, H. K. (1990). *Entomopathogenic Nematodes in Biological Control*. CRC Press, Boca Raton.

Georgis, R. and Gaugler, R. (1991). Predictability in biological control using entomopathogenic nematodes. J. Econ. Entomol. **84**, 713-20.

Georgis, R. and Poinar, G. O., Jr. (1983a). Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). J. Nematol. **15**, 308-11.

Georgis, R. and Poinar, G. O., Jr. (1983b). Effect of soil texture on the distribution and infectivity of *Neoaplectana glaseri* (Nematoda: Steinernematidae). J. Nematol. **15**, 329-32.

Grewal, P. S., Gaugler, R. and Selvan S. (1993). Host recognition by entomopathogenic nematodes: Behavioural response to contact with host feces. J. Chem. Ecol. **17**, 1217-29.

Grewal, P. S., Lewis, E. E. and Gaugler, R. (1995). Evidence for reproductive isolation in a non-reproductive infective stage of entomopathogenic nematodes (Rhabditida: Steinernematidae). Evolution (in press).

Grewal, P. S., Lewis, E. E., Gaugler, R. and Campbell, J. F. (1994). Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. Parasitology **108**, 207-215.

Grewal, P. S. and Wright, D. J. (1992). Migration of *Caenorhabditis elegans* (Nematoda: Rhabditidae) larvae towards bacteria and the nature of bacterial stimulus. Fundam. appl. Nematol. **15**, 159-66.

Hassel, M. P. and Southwood, T. R. E. (1978). Foraging strategies of Insects. Ann. Rev. Ecol. Syst. **9**, 75-98.

Huey, R. B. and Pianka, E. R. (1981). Ecological consequences of foraging mode. Ecology **52**, 991-99.

Ishibashi, N. and Kondo, E. (1990). Behavior of infective juveniles. In: Gaugler, R. and Kaya, H. K. (Eds.) *Entomopathogenic Nematodes in Biological Control* , pp. 139-150. CRC Press, Boca Raton.

Kaya, H. K. (1990). Soil ecology. In: Gaugler, R. and Kaya, H. K. (Eds.) *Entomopathogenic Nematodes in Biological Control* , pp. 93-117. CRC Press, Boca Raton.

Kaya, H. K., Burlando, T. M., and Thurston, G. S. (1993). Two entomopathogenic nematode species with different search strategies for insect suppression. Environ. Entomol. **22**, 859-864.

Kaya, H. K. and Gaugler, R. (1993). Entomopathogenic nematodes. Ann. Rev. of Entomol. **38**, 181-206.

Kennedy, J. S. (1978). The concepts of olfactory 'arrestment' and 'attraction'. Physiol. Entomol. **3**, 91-98.

Kondo, E. and Ishibashi, N. (1986). Nictating behaviour and infectivity of entomogenous nematodes, *Steinernema* spp., to the larvae of the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), on the soil surface. Appl. Ent. Zool. **21**, 553-560.

Lewis, E. E., Gaugler, R. and Harrison, R. (1992). Entomopathogenic nematode host finding: response to host contact cues by cruise and ambush foragers. Parasitology **105**, 103-107.

Lewis, E. E., Gaugler, R. and Harrison, R. (1993). Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Canadian J. Zool. **71**, 765-769.

Lewis, E. E., Grewal, P. S. and Gaugler, R. (1995). Hierarchical orders of host cues in parasite foraging strategies. Parasitology (in press).

Moyle, P. L. and Kaya, H. K. (1981). Dispersal and infectivity of the entomopathogenous nematode, *Neoaplectana carpocapsae* Weiser (Rhabditida: Steinernematidae), in sand. J. Nematol. **13**, 295-300.

O'Brian, W. J., Browman, H. I., and Evans, B. I. (1990). Searching strategies of foraging animals. Amer. Sci. **78**, 152-160.

Poinar, G. O. Jr. (1979). *Nematodes for Biological Control of Insects*. CRC Press, Boca Raton.

Poinar, G. O. Jr. (1990). Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: Gaugler, R. and Kaya, H. K. (Eds.) *Entomopathogenic Nematodes in Biological Control*, pp. 23-61. CRC Press, Boca Raton.

Reed, E. M. and Came, P. B. (1967). The suitability of a nematode (DD-136) for the control of some pasture insects. J. Invertebr. Pathol. **9**, 196-204.

Reed, E. M. and Wallace, H. R. (1965). Leaping locomotion by an insect-parasitic nematode. Nature **206**, 210-211.

Schmidt, J. and All, J. N. (1978). Chemical attraction of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) to insect larvae. Environ. Entomol. **7**, 605-607.

Schmidt, J. and All, J. N. (1979). Attraction of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) to common excretory products of insects. Environ. Entomol. **8**, 55-61.

Schroeder, W. J. and Beavers, J. B. (1987). Movement of entomopathogenous nematodes of the families Heterorhabditidae and Steinernematidae in soil. J. Nematol. **19**, 257-259.

HOST DISCRIMINATION BY *STEINERNEMA CARPOCAPSAE* (WEISER) ALL STRAIN

Manuele Ricci [1], Edwin E. Lewis[2] and Randy Gaugler[2]

[1]Ecogen Europe S.r.l., Pantalla di Todi 06050, Perugia (PG), Italy
[2] Department of Entomology, Rutgers University, New Brunswick, New Jersey 0890-0231, USA.

Entomopathogenic nematode infective juveniles (IJs) or dauers locate and parasitize potential hosts. The success of this process depends on the suitability of the host. The point at which host discrimination occurs, however, remains difficult to determine for most entomopathogenic nematode species. For *Steinernema carpocapsae,* a nematode that forages primarily by ambushing passing hosts, the series of steps leading to parasitization is relatively predictable. Most individuals of this species forage by nictating. Because they rely on the host's movement for contact, the nematodes respond poorly to remote host cues, such as CO_2, when compared with species with cruising search strategies (Lewis *et al.,* 1993). If *S. carpocapsae* enters the haemocoel via the spiracles, contact with host cuticle should cause *S. carpocapsae* IJs to be attracted to CO_2. Previous research showed that cuticle significantly increased *S. carpocapsae* attraction to CO_2 and that nematodes that were stimulated by host contact penetrated into the host haemocoel faster than non-stimulated nematodes (Lewis *et al.,* unpublished). Apparently, host recognition occurs sometime during *S. carpocapsae's* initial exposure to insect cuticle.

We compared the level of stimulation caused by 12 potential hosts, including 3 Lepidoptera, 4 Coleoptera, 1 Orthoptera, 1 Blattodea, 1 Diptera and 2 non-insect arthropods. We also asked whether arthropods that stimulate a high level of attraction by IJs do indeed serve as adequate hosts by testing four parameters of suitability: 1. nematode induced mortality, 2. nematode invasion rate and 3. reproductive potential.

Steinernema carpocapsae was differentially stimulated to be attracted to *G.alleria mellonella* volatiles by contact with the cuticle of different arthropods. *Agrotis ipsilon* pupa, *Leptinotarsa decemlineata, Blattella germanica, Musca domestica,* an Isopod and a Diplopod were not significantly different from the controls. *A. ipsilon* larva, *Tenebrio molitor, Acheta domesticus, G. mellonella, Diabrotica virgifera virgifera* and *Popilia japonica* induced a significantly higher "activation" to *S. carpocapsae* IJs compared to the controls. It seems that *S. carpocapsae* can discriminate even among different stages of the same species. In fact, the value for *A. ipsilon* larva is more than twice that for the pupa.

The same hosts that induced the higher attraction to the volatiles were more susceptible to the nematodes, and the nematodes established better in these arthropods.

Reference

Lewis, E.E., Gaugler, R. and Harrison, R. (1993). Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Can. J. Zool. **71**, 765-769.

DISPERSAL OF INFECTIVE JUVENILES OF *HETERRHABDITIS* SP. UK211 IN THE PRESENCE OF INSECT CADAVERS

Siobhan M. O'Shea, Martin J. Downes and Christine T. Griffin

Department of Biology, St. Patrick's College, Maynooth, Co. Kildare, Ireland.

Pye and Burman (1981) suggested that infective juveniles (dauers) of entomopathogenic nematodes might be stimulated to disperse from the host cadaver by substances emanating from it. We tested this hypothesis using *Heterorhabditis* sp. UK211, an isolate of the North-West European Group. Migration of infective juveniles was tested in sand columns in the presence or absence of a cadaver of *Galleria mellonella*, which had beeen infected with UK211 three weeks previously.

The experiments did not provide evidence in support of the hypothesis. On the contrary, in the presence of a cadaver, *Heterorhabditis* infective juveniles dispersed at a slightly reduced rate. The effect was transient, and was most pronounced after four hours. These findings confirm the results (unpublished) of agar plate assays in our laboratory. The present experiments further suggest that older nematodes are less responsive to the cadaver than younger ones.

Reference

Pye, A.E. and Burman, M. (1981). *Neoaplectana carpocapsae*: nematode accumulations on chemical and bacterial gradients. Exp. Parasitol. **51**, 13-20.

EFFECT OF METAL IONS ON *HETERORHABDITIS BACTERIOPHORA*

Magdalena Jaworska[1], Anna Gorczyca[1], Jadwiga Sepioł[2], and Piotr Tomasik[2]

[1]Department of Entomology and [2]Department of Chemistry and Physics, The Hugon Kołłątaj Academy of Agriculture, Mickiewicz Ave., 24/28, 30-059 Cracow, Poland.

Many biotic and abiotic soil components affect nematode activity, dispersal and host finding; among them are metal ions. Very little experimental work, mainly for particular species of free-living or plant parasitic nematodes, has been done on the subject. The effect of heavy metal ions on nematodes in soil was reported by Bissesar (1982) and Hodoba and Nicholas (1986).The influence of sixteen metal ions: Al, Cd, Co (II), Cr (III), Cr (VI), Cu (II), Fe (III), Li, Mg, Mn (II), Mo (VI), Ni (II), Pb (II), Se (IV), V (V) and Zn on the mortality and infectivity of *Steinemema carpocapsae* under laboratory conditions was previously recognized (Jaworska *et al.*, in press). The present results concern the effect of these same metal ions on *Heterorhabditis bacteriophora.* Metal ions under laboratory investigation were chosen based on the results of ecological monitoring at some major industrial centers in Poland (Curzydło,1988).

The activity of infective juveniles (dauers) of *H. bacteriophora* exposed to the various metal ions differed after 96 hour exposure. Generally, the infective juveniles were rather insensitive to the chemicals regardless of concentration. Solely Pb (II) appeared to be toxic. The mortality of nematodes exposed to some metal ions (e. g. Li, Mg, Mo, Mn) did not exceed that of the control samples and sometimes it was lower. This suggests some protective activity of these ions on the nematodes.

The mortality of wax moth (*Galleria mellonella*) larvae infected by metal-treated entomopathogenic nematodes was generally reduced compared with that caused by non-treated nematodes. The treatment with Co (II), Cr (III), Fe (III), Mo (II), Ni (II), Pb (II) and V (V) significantly ($P < 0.05$) decreased the infectivity of nematodes. Therefore the role of environmental pollution with metals should not be completely ignored.

References

Bissesar, S. (1982). Effect of heavy metals on microorganisms in soils near a secondary lead smelter. Water, Air, Soil Pollut. **17**, 305-308.

Curzydło, J. (1988). Zawartość metali ciężkich w glebach i roślinach uprawnych w rejonie kombinatu metalutgicznego Huta im. Lenina. Agr. Silv., Ser. Agr. **27**, 119-122.

Hodoba, M. and Nicholas, W. L. (1986). Nematode diversity and industrial pollution in the Hunter River Estuary, New South Wales, Australia. Mar. Pollut. Bull. **17**, 251-260.

Jaworska, M., Sepioł, J. and Tomasik, P. (in press). Effect of metal ions under laboratory conditions on the entomopathogenic nematodes *Steinernema carpocapsae (Rhabditida: Steinernematidae).* Water, Air, Soil Pollut.

A NEW WATER DISPERSIBLE GRANULAR FORMULATION OF ENTOMOPATHOGENIC NEMATODES

Parwinder S. Grewal, Ramon Georgis and Bruce Dunlop

biosys, 1057 East Meadow Circle, Palo Alto, CA 94303, USA.

Limited shelf-life and lack of user-frlendly formulations seriously restrict the control potential of entomopathogenic nematodes. The available formulations have enabled the use of nematodes in the medium to high value crops. Generally large volumes of formulated material and tedious preparation steps render nematode products unattractive to traditional low-value agricultural crops. Biosys has developed a new water dispersible granular formulation (WDG) in which large numbers of partially desiccated nematodes are held in small granules. In the granules, nematode energy burn-rate is significantly reduced, extending the shelf-life of *Steinernema carpocapsae* to 5-6 months at room temperatures.

A NEW STRAIN OF *STEINERNEMA ANOMALI* (Kozodoi, 1984) FROM SPAIN

F. García del Pino and A. Palomo

Departamento de Biología Animal, Facultad de Ciencias, Universidad Autónoma de Barcelona 08193 Bellaterra, Barcelona, Spain.

Steinernema anomali was described by Kozodoi (1984) from soil and larvae of *Anomala dubia* Scop. (Col. Scarabeidae), in the Russian provinces of Riazan and Voronez. Up till now, *S. anomali* has been reported only from this central European region. We have isolated a new strain of *S. anomali* from soil samples ("*Galleria* trap" method) of a cherry orchard from Sierra de la Peña de Francia, Salamanca, Spain.

The nematodes were propagated in *Galleria mellonella* larvae. Infective juveniles, and males and females of both generations were killed in warm water, fixed and mounted for observations in light and scanning electron microscopy. Crossing experiments were performed by using the *Galleria* injection method (Akhurst and Bedding, 1979).

Morphological studies show that quantitative measurements of infective juveniles and adults are too variable to be used for differentiating between *S. anomali* and the similar species *S. glaseri*. Poinar and Kozodoi (1988) suggested that the distance from the head to the excretory pore could be used as a diagnostic character for the infective stage of this species. Nevertheless, concerning this character, our *S. anomali* strain overlaps with *S. glaseri*. So the relative position of the excretory pore in relation to the pharynx length (Ratio D), is the only feature that can be used as a diagnostic character. The males only have one constant character, i.e. the spicule tip swollen. We agree with Poinar and Kozodoi (1988) that this is the only consistent morphological difference to separate the adults of this species.

These morphological characters together with the fertile F1 populations obtained in the interbreeding tests, prove that this spanish strain is conspecific with the Russian strain of *S. anomali*. Notwithstanding, several differences between these two strains can be suggested from the low success rate (1.14%) in these inter-population crosses.

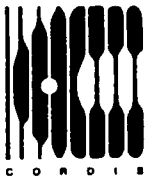
The isolation of this new strain of *S. anomali* from Spain extends the geographical range of this species to southern Europe.

References

Akhurst, R.J. and Bedding, R.A. (1979). The use of a simple cross-breeding technique to facilitate species determination in the genus *Neoaplectana*. Nematologica **24**, 328-330.

Kozodoi, E.M. (1984). A new entomopathogenic nematode, *Neoaplectana anomali* sp. n. (Rhabditida, Steinernematidae) and observations on its biology. Zoolog. Zhurnal **63**, 1605-1609.

Poinar, G.O. and Kozodoi, E.M. (1988). *Neoaplectana glaseri* and *N. anomali* : sibling species or parallelism? Rev. Nématol. **11**, 13-19.



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EUR 16269 – Ecology and transmission strategies of entomopathogenic nematodes

C.T. Griffin, R.L. Gwynn, J.P. Masson

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This volume contains the proceedings of a workshop held in Debrecen, Hungary, from 3-5 June, organized under the auspices of COST 819.

Entomopathogenic nematodes (EPN; *Steinernema* spp and *Heterorhabditis* spp) are insect parasitic nematodes that are mutualistically associated with insect-pathogenic bacteria. A free-living stage, found normally in soil, actively seeks out and enters insects, resulting in host death. EPN are mass-produced and used in inundative application for the control of insect pests. Contributions in this volume set forth a theoretical background in areas of parasitology, ecology and evolutionary biology, that are of relevence to the field of EPN ecology, while others address more specific issues within this field at the level of theory and experiment.

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